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 NEWS  3  May 12  EXTEND option available in structure searching
 NEWS  4  May 12  Polymer links for the POLYLINK command completed in REGISTRY
 NEWS  5  May 27  New UPM (Update Code Maximum) field for more efficient patent
                  SDIs in CAplus
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                  fields
 NEWS 12  AUG 02  CAplus and CA patent records enhanced with European and Japan
                  Patent Office Classifications
 NEWS 13  AUG 02  STN User Update to be held August 22 in conjunction with the
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               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
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FILE 'HOME' ENTERED AT 16:29:01 ON 05 AUG 2004
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=> file medline biosis embase caplus wpids
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FILE 'MEDLINE' ENTERED AT 16:29:27 ON 05 AUG 2004

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=> ((Shiga (w) like) or Shiga) (s) toxin
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=> s ((Shiga (w) like) or Shiga) (s) toxin
L1          7735 ((SHIGA (W) LIKE) OR SHIGA) (S) TOXIN

=> s l1 and mutat? (s) (B or binding)
L2            67 L1 AND MUTAT? (S) (B OR BINDING)

=> dup rem
ENTER L# LIST OR (END):l2
PROCESSING COMPLETED FOR L2
L3            37 DUP REM L2 (30 DUPLICATES REMOVED)

=> t ti l3 1-37

L3   ANSWER 1 OF 37  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
TI   Effects of HIV-1 Nef on retrograde transport from the plasma membrane to
     the endoplasmic reticulum.

L3   ANSWER 2 OF 37  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
TI   Mutations in hns reduce the adherence of Shiga toxin
     -producing E. coli 091:H21 strain B2F1 to human colonic epithelial cells
     and increase the production of hemolysin.

L3   ANSWER 3 OF 37  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   Immunogenic composition, useful to prevent or treat pathogenic bacterial
     infection, comprises live bacteria with DNA adenine methylase activity
     altered relative to wild-type, and which also express a heterologous
     antigen.

L3   ANSWER 4 OF 37     MEDLINE on STN                    DUPLICATE 1
TI   A mutational analysis of the globotriaosylceramide-
     binding sites of verotoxin VT1.
```

L3 ANSWER 5 OF 37 MEDLINE on STN
TI Development of vaccine for enterohemorrhagic Escherichia coli infection.

L3 ANSWER 6 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Enterohemolysin operon of **Shiga toxin**-producing Escherichia coli: A virulence function of inflammatory cytokine production from human monocytes.

L3 ANSWER 7 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2
TI AB5 toxin B subunit mutants with altered chemical conjugation characteristics

L3 ANSWER 8 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
TI Chimeric nontoxic mutants of enterotoxins as mucosal adjuvants for cell-mediated or humoral immunity

L3 ANSWER 9 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
TI Mutated anthrax toxin protective antigen proteins that specifically target cells containing high amounts of cell-surface metalloproteinases or plasminogen activator receptors

L3 ANSWER 10 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Differences in levels of secreted locus of enterocyte effacement proteins between human disease-associated and bovine Escherichia coli O157.

L3 ANSWER 11 OF 37 MEDLINE on STN DUPLICATE 3
TI Probing the surface of eukaryotic cells using combinatorial toxin libraries.

L3 ANSWER 12 OF 37 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Mutations in the csgD promoter of E. coli O157:H7 associated with increased virulence in mice and increased invasion of HEp-2 cells.

L3 ANSWER 13 OF 37 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 4
TI Construction of deletion mutants of **Shiga** (**-like**) **toxin** genes (stx-1 and/or stx-2) on enterohemorrhagic Escherichia coli (O157: H7).

L3 ANSWER 14 OF 37 MEDLINE on STN DUPLICATE 5
TI Genetic analysis for virulence factors in Escherichia coli O104:H21 that was implicated in an outbreak of hemorrhagic colitis.

L3 ANSWER 15 OF 37 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI Protein transduction system for treating cancer and pathogenic infections has a fusion protein comprising a protein transduction domain covalently linked to a cytotoxic domain.

L3 ANSWER 16 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI A mutant **Shiga-like toxin** lie bound to its receptor Gb3: Structure of a group II **Shiga-like toxin** with altered binding specificity.

L3 ANSWER 17 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Production of **Shiga toxin** by Escherichia coli measured with reference to the membrane vesicle-associated toxins.

L3 ANSWER 18 OF 37 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN

TI Production of cytotoxic heteromeric protein combinatorial libraries, useful for ability to specifically bind to and kill a target cell.

L3 ANSWER 19 OF 37 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI New chimeric constructs of **Shiga toxin** B fragment with polypeptide or nucleic acid - to provide retrograde transport in cells, particularly for presentation of antigenic epitopes or for restoration of defective intracellular transport.

L3 ANSWER 20 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Proteolytic cleavage of the A subunit is essential for maximal cytotoxicity of Escherichia coli O157:H7 **Shiga-like toxin**-1.

L3 ANSWER 21 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Identification and characterization of a newly isolated **shiga toxin** 2- converting phage from **shiga toxin** -producing Escherichia coli.

L3 ANSWER 22 OF 37 MEDLINE on STN DUPLICATE 6
TI Modeling the carbohydrate-binding specificity of pig edema toxin.

L3 ANSWER 23 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Role of the disulfide bond in **shiga toxin** A-chain for **toxin** entry into cells.

L3 ANSWER 24 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Investigation of ribosome binding by the **shiga toxin** A1 subunit, using competition and site-directed mutagenesis.

L3 ANSWER 25 OF 37 MEDLINE on STN DUPLICATE 7
TI Phenylalanine 30 plays an important role in receptor binding of verotoxin-1.

L3 ANSWER 26 OF 37 MEDLINE on STN DUPLICATE 8
TI Two distinct binding sites for globotriaosyl ceramide on verotoxins: identification by molecular modelling and confirmation using deoxy analogues and a new glycolipid receptor for all verotoxins.

L3 ANSWER 27 OF 37 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Determination of a second **binding** site in the **Shiga-like toxin** family **binding** subunits by **mutational** analysis.

L3 ANSWER 28 OF 37 MEDLINE on STN DUPLICATE 9
TI Analysis of **Shiga toxin** subunit association by using hybrid A polypeptides and site-specific mutagenesis.

L3 ANSWER 29 OF 37 MEDLINE on STN DUPLICATE 10
TI Modelling of the interaction of verotoxin-1 (VT1) with its glycolipid receptor, globotriaosylceramide (Gb3).

L3 ANSWER 30 OF 37 MEDLINE on STN DUPLICATE 11
TI Alteration of the glycolipid binding specificity of the pig edema toxin from globotetraosyl to globotriaosyl ceramide alters in vivo tissue targetting and results in a verotoxin 1-like disease in pigs.

L3 ANSWER 31 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.

on STN
TI Proteolytic cleavage at arginine residues within the hydrophilic disulphide loop of the Escherichia coli **Shiga-like toxin** I A subunit is not essential for cytotoxicity.

L3 ANSWER 32 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Identification of a B subunit gene promoter in the **Shiga toxin** operon of Shigella dysenteriae 1.

L3 ANSWER 33 OF 37 CAPLUS COPYRIGHT 2004 ACS on STN
TI Construction of stable LamB-**Shiga toxin** B subunit hybrids: analysis of expression in Salmonella typhimurium aroA strains and stimulation of B subunit-specific mucosal and serum antibody responses

L3 ANSWER 34 OF 37 MEDLINE on STN DUPLICATE 12
TI Alteration of the carbohydrate binding specificity of verotoxins from Gal alpha 1-4Gal to GalNAc beta 1-3Gal alpha 1-4Gal and vice versa by site-directed mutagenesis of the binding subunit.

L3 ANSWER 35 OF 37 MEDLINE on STN DUPLICATE 13
TI Identification of three amino acid residues in the B subunit of **Shiga toxin** and **Shiga-like toxin** type II that are essential for holotoxin activity.

L3 ANSWER 36 OF 37 MEDLINE on STN DUPLICATE 14
TI Functional analysis of the **Shiga toxin** and **Shiga-like toxin** type II variant binding subunits by using site-directed mutagenesis.

L3 ANSWER 37 OF 37 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Iron regulation of **Shiga-like toxin** expression of Escherichia coli is mediated by the fur locus.

=> d his

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FILE 'MEDLINE, BIOSIS, EMBASE, CAPLUS, WPIDS' ENTERED AT 16:29:27 ON 05 AUG 2004
L1 7735 S ((SHIGA (W) LIKE) OR SHIGA) (S) TOXIN
L2 67 S L1 AND MUTAT? (S) (B OR BINDING)
L3 37 DUP REM L2 (30 DUPLICATES REMOVED)

=> s l3 and resistan?
L4 2 L3 AND RESISTAN?

=> t ti l4 1-2

L4 ANSWER 1 OF 2 MEDLINE on STN
TI Probing the surface of eukaryotic cells using combinatorial toxin libraries.

L4 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Construction of deletion mutants of **Shiga** (**-like**) **toxin** genes (stx-1 and/or stx-2) on enterohemorrhagic Escherichia coli (O157: H7).

=> d ibib abs l4 1-2

L4 ANSWER 1 OF 2 MEDLINE on STN
ACCESSION NUMBER: 2001272582 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11369233
TITLE: Probing the surface of eukaryotic cells using combinatorial toxin libraries.
AUTHOR: Bray M R; Bisland S; Perampalam S; Lim W M; Gariepy J
CORPORATE SOURCE: Ontario Cancer Institute, Princess Margaret Hospital Rm. 7-117, 610 University Avenue, Ontario, M5G 2M9, Toronto, Canada.
SOURCE: Current biology : CB, (2001 May 1) 11 (9) 697-701. Journal code: 9107782. ISSN: 0960-9822.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010716
Last Updated on STN: 20010716
Entered Medline: 20010712

AB The success of proteomics hinges in part on the development of approaches able to map receptors on the surface of cells. One strategy to probe a cell surface for the presence of internalized markers is to make use of **Shiga-like toxin** 1 (SLT-1), a ribosome-inactivating protein that kills eukaryotic cells [1, 2]. SLT-1 binds to the glycolipid globotriaosylceramide [3, 4], which acts as a shuttle, allowing the toxin to be imported and routed near ribosomes. We investigated the use of SLT-1 as a structural template to create combinatorial libraries of toxin variants with altered receptor specificity. Since all SLT-1 variants retain their toxic function, this property served as a search engine enabling us to identify mutants from these libraries able to kill target cells expressing internalizable receptors. Random **mutations** were introduced in two discontinuous loop regions of the SLT-1 receptor **binding** subunit. Minimal searches from screening 600 bacterial colonies randomly picked from an SLT-1 library identified toxin mutants able to kill cell lines **resistant** to the wild-type toxin. One such mutant toxin was shown to bind to a new receptor on these cell lines by flow cytometry. Toxin libraries provide a strategy to delineate the spectrum of receptors on eukaryotic cells.

L4 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:211149 BIOSIS
DOCUMENT NUMBER: PREV200300211149
TITLE: Construction of deletion mutants of **Shiga** (-**like**) **toxin** genes (stx-1 and/or stx-2) on enterohemorrhagic Escherichia coli (O157: H7).
AUTHOR(S): Yokoyama, Shin-Ichiro [Reprint Author]; Suzuki, Tohru; Shiraishi, Shuichi; Ohishi, Nobuko; Yagi, Kunio; Ichihara, Shigeyuki; Itoh, Saori; Mori, Hiroshi
CORPORATE SOURCE: Laboratory of Microbiology, Department of Public Health Pharmacy, Gifu Pharmaceutical University, 5-6-1 Mitahora-Higashi, Gifu, 502-8585, Japan
yokoyama@gifu-pu.ac.jp
SOURCE: Journal of Clinical Biochemistry and Nutrition, (2001) Vol. 30, pp. 33-42. print.
CODEN: JCBNER. ISSN: 0912-0009.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Apr 2003
Last Updated on STN: 30 Apr 2003

AB We constructed isogenic **Shiga** (-**like**) **toxin**

(Stx-1 and/or Stx-2) gene-deletion mutants of enterohemorrhagic Escherichia coli (EHEC) GPU96MM (O157: H7). A vector with temperature-sensitive replication origin was used for the construction. The parts of stx-1 and stx-2 on the GPU96MM genome were replaced with kanamycin and chloramphenicol **resistance** genes, respectively. The mutants deficient in stx-1, stx-2, and both of them were named GPU993, GPU994, and GPU995, respectively. Each **mutation** was confirmed by the polymerase chain reaction, enzyme-linked immunosorbent assay using antibodies to **B** subunits of Stx-1 and Stx-2, and the cytotoxic activity of the bacterial culture supernatants toward HeLa cells was detected for GPU96MM and the mutants except for GPU995. These results indicate that GPU993 and GPU994 lack productivity for the respective toxins and GPU995, for both of them.

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=> s l4 not l3
L5            0 L4 NOT L3

=> s l3 not l4
L6           35 L3 NOT L4

=> d ibib abs l6 1-35

L6   ANSWER 1 OF 35     MEDLINE on STN
ACCESSION NUMBER:   2002335753     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 12078077
TITLE:              Development of vaccine for enterohemorrhagic Escherichia
                    coli infection.
AUTHOR:             Yamasaki Shinji
CORPORATE SOURCE:   Department of Veterinary Sciences, Graduate School of
                    Agriculture and Biological Sciences, Osaka Prefecture
                    University.
SOURCE:             Nippon rinsho. Japanese journal of clinical medicine, (2002
                    Jun) 60 (6) 1083-8.  Ref: 22
                    Journal code: 0420546. ISSN: 0047-1852.
PUB. COUNTRY:       Japan
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
                    General Review; (REVIEW)
                    (REVIEW, TUTORIAL)
LANGUAGE:           Japanese
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200208
ENTRY DATE:         Entered STN: 20020625
                    Last Updated on STN: 20020803
                    Entered Medline: 20020802
AB   Efforts on the development of vaccines against enterohemorrhagic
     Escherichia coli (EHEC) infection has been described in this review.  Two
     kinds of vaccines were developed and these have been targeted for in
     humans and cattle.  One vaccine candidate is toxoid, which uses an
     inactive form of Shiga toxin(Stx).  A part of
     B subunit, each B or A subunit or one or two amino acid
     mutated holotoxin were developed as a toxoid vaccine candidate.
     The other candidate was bacterial surface antigen such as a live
     attenuated EHEC and hybrid between non-toxic LPS and toxoid.  A live
     attenuated vaccine against EHEC O26: H11, O157: H7, O139: H1 were
     developed.  Further a live attenuated vaccine candidate of Vibrio cholerae
     O1 expressing Stx1-B, Shigella flexneri expressing S. dysenteriae
     O-antigen and Stx1-B, or Salmonella Typhimurium expressing O111 antigen
     were developed.  Hybrid type vaccine candidates were also developed with
     O111 LPS and tetanus toxoid, O157 LPS and exotoxin, and O157 LPS and
     Stx1-B.
```

L6 ANSWER 2 OF 35 MEDLINE on STN
ACCESSION NUMBER: 2002120889 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11723119
TITLE: A **mutational** analysis of the globotriaosylceramide-**binding** sites of verotoxin VT1.
AUTHOR: Soltyk Anna M; MacKenzie C Roger; Wolski Vince M; Hirama Tomoko; Kitov Pavel I; Bundle David R; Brunton James L
CORPORATE SOURCE: Clinical Science Division, University of Toronto, Toronto, Ontario M5S 1A8, Canada.
SOURCE: Journal of biological chemistry, (2002 Feb 15) 277 (7) 5351-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20020222
Last Updated on STN: 20030105
Entered Medline: 20020321

AB Escherichia coli verotoxin, also known as **Shiga-like toxin**, binds to eukaryotic cell membranes via the glycolipid Gb(3) receptors which present the P(k) trisaccharide Galalpha(1-4)Galbeta(1-4)Glcbeta. Crystallographic studies have identified three P(k) trisaccharide (P(k)-glycoside) binding sites per verotoxin 1B subunit (VT1B) monomer while NMR studies have identified binding of P(k)-glycoside only at site 2. To understand the basis for this difference, we studied binding of wild type VT1B and VT1B mutants, defective at one or more of the three sites, to P(k)-glycoside and pentavalent P(k) trisaccharide (pentaSTARFISH) in solution and Gb(3) presented on liposomal membranes using surface plasmon resonance. Site 2 was the key site in terms of free trisaccharide binding since mutants altered at sites 1 and 3 bound this ligand with wild type affinity. However, effective binding of the pentaSTARFISH molecule also required a functional site 3, suggesting that site 3 promotes pentavalent binding of linked trisaccharides at site 1 and site 2. Optimal binding to membrane-associated Gb(3) involved all three sites. Binding of all single site mutants to liposomal Gb(3) was weaker than wild type VT1B binding. Site 3 mutants behaved as if they had reduced ability to enter into high avidity interactions with Gb(3) in the membrane context. Double mutants at site 1/site 3 and site 2/site 3 were completely inactive in terms of binding to liposomal Gb(3,) even though the site 1/site 3 mutant bound trisaccharide with almost wild type affinity. Thus site 2 alone is not sufficient to confer high avidity binding to membrane-localized Gb(3). Cytotoxic activity paralleled membrane glycolipid binding. Our data show that the interaction of verotoxin with the Gb(3) trisaccharide is highly context dependent and that a membrane environment is required for biologically relevant studies of the interaction.

L6 ANSWER 3 OF 35 MEDLINE on STN
ACCESSION NUMBER: 2001131078 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11136742
TITLE: Genetic analysis for virulence factors in Escherichia coli O104:H21 that was implicated in an outbreak of hemorrhagic colitis.
AUTHOR: Feng P; Weagant S D; Monday S R
CORPORATE SOURCE: Division of Microbiological Studies, Food and Drug Administration, Washington, DC 20204, USA..
pfeng@cfsan.fda.gov
SOURCE: Journal of clinical microbiology, (2001 Jan) 39 (1) 24-8.
Journal code: 7505564. ISSN: 0095-1137.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20021008
Entered Medline: 20010301

AB Isolates of enterohemorrhagic Escherichia coli (EHEC) of serotype O104:H21 implicated in a 1994 outbreak of hemorrhagic colitis in Montana were analyzed for the presence of trait EHEC virulence markers. By using a multiplex PCR that specifically amplifies several genes, the O104:H21 strains were found to carry only the **Shiga toxin** 2 gene (stx2) and to express Stx2. They did not have the eaeA gene for gamma-intimin, which is typically found in O157:H7, or the alpha- or beta-intimin derivatives, which are common in other EHEC and enteropathogenic E. coli serotypes. Results of the multiplex PCR also indicated that the ehxA gene for enterohemolysin was absent from O104:H21. This, however, was not consistent with the results of a phenotypic assay that showed them to be hemolytic or a PCR analysis with another set of ehxA-specific primers, which indicated the presence of ehxA. To resolve this discrepancy, the ehxA region in O104:H21 and O157:H7 strains, to which the multiplex PCR primers anneal, was cloned and sequenced. Comparison of the sequences showed that the upstream primer binding site in the ehxA gene of O104:H21 was not identical to that of O157:H7. Specifically, there were several base **mutations**, including an A-to-G substitution at the 3' end of the primer **binding** site. These base mutations are presumably not unique to O104:H21, since other enterohemolytic serotypes were also not detected with the ehxA primers used in the multiplex PCR. Comparison of the ehxA sequences of O104:H21 strains with those of other Stx-producing E. coli strains showed that they more closely resembled those of O8:H19 strains, which have cluster II ehxA genes, than those of O157:H7 strains, which have cluster I ehxA sequences. By modifying the upstream ehxA primer, the multiplex PCR was able to detect ehxA genes in both O157:H7 and O104:H21 strains.

L6 ANSWER 4 OF 35 MEDLINE on STN
ACCESSION NUMBER: 1998153656 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9485304
TITLE: Modeling the carbohydrate-binding specificity of pig edema toxin.
AUTHOR: Cummings M D; Ling H; Armstrong G D; Brunton J L; Read R J
CORPORATE SOURCE: Department of Biochemistry, University of Alberta, Edmonton, Canada.
SOURCE: Biochemistry, (1998 Feb 17) 37 (7) 1789-99.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980326
Last Updated on STN: 19980326
Entered Medline: 19980317

AB The wild-type binding pentamer of **Shiga-like toxin** IIe (SLT-IIe) binds both the globotriaosylceramide (Gb3) and globotetraosylceramide (Gb4) cell surface glycolipids, whereas the double mutant GT3 (Q65E/K67Q) exhibits a marked preference for Gb3 [Tyrrell, G. J., et al. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 524-528]. We modeled three unique sites (sites 1-3) for binding of the carbohydrate moiety of Gb3 to GT3 and SLT-IIe, on the basis of the three sites observed for the SLT-I pentamer [Ling, H., et al. (1998) Biochemistry 37,

1777-1788]. Examination of the three sites in light of various **mutation** and **binding** data strongly suggested that one of the **binding** sites plays a role in the change of specificity observed for the GT3 mutant. We applied several modeling techniques, and developed a model for binding of the carbohydrate moiety of Gb4 to this site of the SLT-IIe binding pentamer. This model is consistent with a wide variety of **mutation** and **binding** data and clearly shows the importance of the terminal GalNAc residue of Gb4, as well as that of the two **mutated** residues of GT3, to the intermolecular interaction.

L6 ANSWER 5 OF 35 MEDLINE on STN
ACCESSION NUMBER: 97113247 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8807854
TITLE: Two distinct binding sites for globotriaosyl ceramide on verotoxins: identification by molecular modelling and confirmation using deoxy analogues and a new glycolipid receptor for all verotoxins.
COMMENT: Erratum in: Chem Biol 1996 Jan;3(1):503
AUTHOR: Nyholm P G; Magnusson G; Zheng Z; Norel R; Binnington-Boyd B; Lingwood C A
CORPORATE SOURCE: Department of Molecular and Medical Genetics, University of Toronto, Ontario, Canada.. cling@sickkids.on.ca
SOURCE: Chemistry & biology, (1996 Apr) 3 (4) 263-75.
Journal code: 9500160. ISSN: 1074-5521.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19980206
Entered Medline: 19970204

AB BACKGROUND: The Escherichia coli verotoxins (VTs) can initiate human vascular disease via the specific recognition of globotriaosyl-ceramide (Gb3) on target endothelial cells. To explore the structural basis for receptor recognition by different VTs we used molecular modelling based on the crystal structure of VT1, **mutational** data and **binding** data for deoxy galabiosyl receptors. RESULTS: We propose a model for the verotoxin 'cleft-site complex' with Gb3. Energy minimizations of Gb3 within the 'cleft site' of verotoxins VT1, VT2, VT2c and VT2e resulted in stable complexes with hydrogen-bonding systems that were in agreement with binding data obtained for mono-deoxy analogues of Gb3. N-deacetylated globoside (aminoGb4), which was found to be a new, efficient receptor for all verotoxins, can be favourably accommodated in the cleft site of the VTs by formation of a salt bridge between the galactosamine and a cluster of aspartates in the site. The model is further extended to explain the binding of globoside by VT2e. Docking data support the possibility of an additional binding site for Gb3 on VT1. CONCLUSIONS: The proposed models for the complexes of verotoxins with their globoglycolipid receptors are consistent with receptor analogue **binding** data and explain previously published **mutational** studies. The results provide a first approach to the design of specific inhibitors of VT-receptor binding.

L6 ANSWER 6 OF 35 MEDLINE on STN
ACCESSION NUMBER: 96417866 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8820657
TITLE: Phenylalanine 30 plays an important role in receptor binding of verotoxin-1.
AUTHOR: Clark C; Bast D; Sharp A M; St Hilaire P M; Agha R; Stein P E; Toone E J; Read R J; Brunton J L

CORPORATE SOURCE: Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada.
SOURCE: Molecular microbiology, (1996 Feb) 19 (4) 891-9.
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961216

AB The homopentameric B subunit of verotoxin 1 (VT1) binds to the glycosphingolipid receptor globotriaosylceramide (Gb3). We produced mutants with alanine substitutions for residues found near the cleft between adjacent subunits. Substitution of alanine for phenylalanine 30 (Phe-30) resulted in a fourfold reduction in B subunit binding affinity for Gb3 and a 10-fold reduction in receptor density in a solid-phase binding assay. The interaction of wild-type and mutant B subunits with Pk trisaccharide in solution was examined by titration microcalorimetry. The carbohydrate binding of the mutant was markedly impaired compared with that of the wild type and was too weak to allow calculation of a binding constant. These results demonstrate that the **mutation** significantly impaired the carbohydrate-**binding** function of the **B** subunit. To ensure that the **mutation** had not caused a significant change in structure, the mutant **B** subunit was crystallized and its structure was determined by X-ray diffraction. Difference Fourier analysis showed that its structure was identical to that of the wild type, except for the substitution of alanine for Phe-30. The mutation was also produced in the VT1 operon, and mutant holotoxin was purified to homogeneity. The cytotoxicity of the mutant holotoxin was reduced by a factor of 10(5) compared to that of the wild type in the Vero cell cytotoxicity assay. The results suggest that the aromatic ring of Phe-30 plays a major role in binding of the B subunit to the Galalpha1-4Galbeta1-4Glc trisaccharide portion of Gb3. Examination of the VT1 B crystal structure suggests two potential carbohydrate-binding sites which lie on either side of Phe-30.

L6 ANSWER 7 OF 35 MEDLINE on STN
ACCESSION NUMBER: 96059592 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7577818
TITLE: Modelling of the interaction of verotoxin-1 (VT1) with its glycolipid receptor, globotriaosylceramide (Gb3).
AUTHOR: Nyholm P G; Brunton J L; Lingwood C A
CORPORATE SOURCE: Department of Molecular and Medical Genetics, University of Toronto, Ontario, Canada.
SOURCE: International journal of biological macromolecules, (1995 Jun) 17 (3-4) 199-204.
Journal code: 7909578. ISSN: 0141-8130.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199511
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19960124
Entered Medline: 19951127

AB Possible **binding** sites for the glycolipid globotriaosylceramide (Gal alpha 1-->4Gal beta 1-->4Glc beta 1-->1 Cer; Gb3) on the **B** -subunits of verotoxin-1 (VT1) were explored using **binding** data for specifically **mutated** verotoxins and by computational docking of favoured conformers of Gb3 with the crystal structure of VT1.

Calculations using the GRID program suggested a site with favourable hydrophobic interactions at the exposed side chain of Phe30. One of the favoured conformers of Gb3 was docked into this site, with the hydrophobic face of the internal Gal beta residue in contact with the side chain of Phe30. After energy minimization, the two terminal saccharide residues of Gb3 (Gal alpha and Gal beta) showed favourable interactions with the toxin. In the proposed model of the complex, the terminal Gal alpha of Gb3 is located in proximity to aspartates 16-18 of VT1. The model is in agreement with available experimental **binding** data for the interaction of globoglycolipids with different naturally occurring and **mutated** verotoxins.

L6 ANSWER 8 OF 35 MEDLINE on STN
ACCESSION NUMBER: 95286493 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7768810
TITLE: Analysis of **Shiga toxin** subunit association by using hybrid A polypeptides and site-specific mutagenesis.
AUTHOR: Jemal C; Haddad J E; Begum D; Jackson M P
CORPORATE SOURCE: Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201, USA.
CONTRACT NUMBER: AI29929 (NIAID)
SOURCE: Journal of bacteriology, (1995 Jun) 177 (11) 3128-32. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950713
Last Updated on STN: 19950713
Entered Medline: 19950706

AB **Shiga toxin** (STX), a bacterial **toxin** produced by Shigella dysenteriae type 1, is a hexamer composed of five receptor-binding B subunits which encircle an alpha-helix at the carboxyl terminus of the enzymatic A polypeptide. Hybrid toxins constructed by fusing the A polypeptide sequences of STX and **Shiga-like toxin** type II were used to confirm that the carboxyl terminus of the A subunits governs association with the B pentamers. The alpha-helix of the 293-amino-acid STX A subunit contains nine residues (serine 279 to methionine 287) which penetrate the nonpolar pore of the B-subunit pentamer. Site-directed mutagenesis was used to establish the involvement of two residues bordering this alpha-helix, aspartic acid 278 and arginine 288, in coupling the C terminus of StxA to the B pentamer. Amino acid substitutions at StxB residues arginine 33 and tryptophan 34, which are on the membrane-contacting surface of the pentamer, reduced cytotoxicity without affecting holotoxin formation. Although these **B**-subunit **mutations** did not involve receptor-**binding** residues, they may have induced an electrostatic repulsion between the holotoxin and the mammalian cell membrane or disrupted cytoplasmic translocation.

L6 ANSWER 9 OF 35 MEDLINE on STN
ACCESSION NUMBER: 93267226 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8496689
TITLE: Alteration of the glycolipid binding specificity of the pig edema toxin from globotetraosyl to globotriaosyl ceramide alters in vivo tissue targetting and results in a verotoxin 1-like disease in pigs.
AUTHOR: Boyd B; Tyrrell G; Maloney M; Gyles C; Brunton J; Lingwood C
CORPORATE SOURCE: Department of Microbiology, Hospital for Sick Children,

Toronto, Ontario, Canada.
SOURCE: Journal of experimental medicine, (1993 Jun 1) 177 (6) 1745-53.
Journal code: 2985109R. ISSN: 0022-1007.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199306
ENTRY DATE: Entered STN: 19930702
Last Updated on STN: 19930702
Entered Medline: 19930621

AB All members of the verotoxin (VT) family specifically recognize globo-series glycolipids on the surface of susceptible cells. Those toxins that are associated with human disease, VT1, VT2, and VT2c, bind to globotriaosyl ceramide (Gb3) while VT2e, which is associated with edema disease of swine, binds preferentially to globotetraosyl ceramide (Gb4). We were recently able to identify, using site-directed mutagenesis, amino acids in the binding subunit of these toxins that are important in defining their glycosphingolipid (GSL) binding specificity (Tyrrell, G. J., K. Ramotar, B. Boyd, B. W. Toye, C. A. Lingwood, and J. L. Brunton. 1992. Proc. Natl. Acad. Sci. USA. 89:524). The concomitant **mutation** of Gln64 and Lys66 in the VT2e **binding** subunit to the corresponding residues (Glu and Gln, respectively) found in VT2 effectively converted the GSL **binding** specificity of the mutant toxin from Gb4 to Gb3 in vitro. We now report that the altered carbohydrate recognition of the mutant toxin (termed GT3) has biological significance, resulting in a unique disease after intravascular injection into pigs as compared with classical VT2e-induced edema disease. The tissue localization of radiolabeled GT3 after intravascular injection was elevated in neural tissues compared with VT2e accumulation, while localization of GT3 to the gastrointestinal tract was relatively reduced. Accordingly, the pathological lesions after challenge with GT3 involved gross edema of the cerebrum, cerebellum, and brain stem, while purified VT2e caused hemorrhage and edema of the cerebellum, and submucosa of the stomach and large intestine. In addition, both radiolabeled toxins bound extensively to tissues not directly involved in the pathology of disease. VT2e, unlike GT3 or VT1, bound extensively to red cells, which have high levels of Gb4. The overall tissue distribution of VT2e was thus found to be influenced by regional blood flow to each organ and not solely by the Gb4 levels of these tissues. Conversely, the distribution of GT3 (and VT1), which cleared more rapidly from the circulation, correlated with respective tissue Gb3 levels rather than blood flow. These studies indicate the primary role of carbohydrate binding specificity in determining systemic pathology, suggest that the red cells act as a toxin carrier in edema disease, and indicate that red cell binding does not protect against the pathology of systemic verotoxemia.

L6 ANSWER 10 OF 35 MEDLINE on STN
ACCESSION NUMBER: 92115693 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1731324
TITLE: Alteration of the carbohydrate binding specificity of verotoxins from Gal alpha 1-4Gal to GalNAc beta 1-3Gal alpha 1-4Gal and vice versa by site-directed mutagenesis of the binding subunit.
AUTHOR: Tyrrell G J; Ramotar K; Toye B; Boyd B; Lingwood C A; Brunton J L
CORPORATE SOURCE: Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1992 Jan 15) 89 (2) 524-8.
Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920308
Last Updated on STN: 19920308
Entered Medline: 19920218

AB Verotoxin 1 (VT-1) and **Shiga-like toxin** II (SLT-II) bind to the glycosphingolipid (GSL), globotriaosylceramide (Gb3), whereas pig edema disease **toxin** (VTE) binds to globotetraosylceramide (Gb4) and to a lesser degree Gb3. Amino acids important in the GSL binding specificity of VT-1 and VTE have been identified by site-directed mutagenesis. One **mutation,** Asp-18----Asn, in VT-1 resulted in **binding** to Gb4 in addition to Gb3 in a manner similar to VTE. Several **mutations** in VTE resulted in the complete loss of GSL **binding;** however, one **mutation** resulted in a change in the GSL **binding** specificity of the VTE **B** subunit. The double **mutation** Gln-64----Glu and Lys-66----Gln (designated GT3) caused a selective loss of Gb4 **binding,** effectively changing the **binding** phenotype from VTE to VT-1. Both wild-type VTE and GT3 were purified to homogeneity and binding kinetics in vitro were determined with purified GSLs from human kidney. The cell cytotoxicity spectrum of the mutant toxin was also found to be altered in comparison with VTE. These changes were consistent with the GSL content of the target cells.

L6 ANSWER 11 OF 35 MEDLINE on STN
ACCESSION NUMBER: 91123188 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1991714
TITLE: Identification of three amino acid residues in the B subunit of **Shiga toxin** and **Shiga-like toxin** type II that are essential for holotoxin activity.
AUTHOR: Perera L P; Samuel J E; Holmes R K; O'Brien A D
CORPORATE SOURCE: Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799.
CONTRACT NUMBER: AI 20148-06 (NIAID)
SOURCE: Journal of bacteriology, (1991 Feb) 173 (3) 1151-60.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910405
Last Updated on STN: 19970203
Entered Medline: 19910311

AB **Shiga toxin** of Shigella dysenteriae type I and **Shiga-like** toxins I and II (SLT-I and SLT-II, respectively) of enterohemorrhagic Escherichia coli are functionally similar protein cytotoxins. These toxin molecules have a bipartite molecular structure which consists of an enzymatically active A subunit that inhibits protein synthesis in eukaryotic cells and an oligomeric B subunit that binds to globotriaosylceramide glycolipid receptors on eukaryotic cells. Regionally directed chemical mutagenesis of the B subunit of SLT-II was used to identify amino acids in the B subunit that are critical for SLT-II holotoxin cytotoxic activity. Three noncytotoxic mutants were isolated, and their mutations were mapped. The substitutions of arginine with cysteine at codon 32, alanine with threonine at codon 42, and glycine with aspartic acid at codon 59 in the 70-amino-acid mature SLT-II B polypeptide resulted in the complete abolition of cytotoxicity.

The analogous arginine, alanine, and glycine residues were conserved at codons 33, 43, and 60 in the 69-amino-acid mature B polypeptide of **Shiga toxin**. Comparable **mutations** induced in the **B**-subunit gene of **Shiga toxin** by oligonucleotide-directed, site-specific mutagenesis resulted in drastically decreased cytotoxicity (10(3)- to 10(6)-fold) as compared with that of wild-type **Shiga toxin**. The mutant SLT-II and **Shiga toxin** B subunits were characterized for stability, receptor binding, immunoreactivity, and ability to be assembled into holotoxin.

L6 ANSWER 12 OF 35 MEDLINE on STN
ACCESSION NUMBER: 90130298 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2404947
TITLE: Functional analysis of the **Shiga toxin** and **Shiga-like toxin** type II variant binding subunits by using site-directed mutagenesis.
AUTHOR: Jackson M P; Wadolkowski E A; Weinstein D L; Holmes R K; O'Brien A D
CORPORATE SOURCE: Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799.
CONTRACT NUMBER: AI 20148-06 (NIAID)
SOURCE: Journal of bacteriology, (1990 Feb) 172 (2) 653-7. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199003
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19970203
Entered Medline: 19900314
AB The B subunit of **Shiga toxin** and the **Shiga-like** toxins (SLTs) mediates receptor binding, cytotoxic specificity, and extracellular localization of the holotoxin. While the functional receptor for **Shiga toxin**, SLT type I (SLT-I), and SLT-II is the glycolipid designated Gb3, SLT-II variant (SLT-IIv) may use a different glycolipid receptor. To identify the domains responsible for receptor binding, localization in Escherichia coli, and recognition by neutralizing monoclonal antibodies, oligonucleotide-directed site-specific mutagenesis was used to alter amino acid residues in the B subunits of **Shiga toxin** and SLT-IIv. Mutagenesis of a well-conserved hydrophilic region near the amino terminus of the **Shiga toxin** B subunit rendered the molecule nontoxic but did not affect immunoreactivity or holotoxin assembly. In addition, elimination of one cysteine residue, as well as truncation of the B polypeptide by 5 amino acids, caused a total loss of activity. Changing a glutamate to a glutamine at the carboxyl terminus of the **Shiga toxin** B subunit resulted in the loss of receptor binding and immunoreactivity. However, the corresponding **mutation** in the SLT-IIv **B** subunit (glutamine to glutamate) did not reduce the levels of cytotoxicity but did affect extracellular localization of the holotoxin in E. coli.

L6 ANSWER 13 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:233509 BIOSIS
DOCUMENT NUMBER: PREV200200233509
TITLE: Mutations in the csgD promoter of E. coli O157:H7 associated with increased virulence in mice and increased invasion of HEp-2 cells.
AUTHOR(S): Uhlich, G. A. [Reprint author]; Keen, J. E.; Elder, R. O.

CORPORATE SOURCE: USDA, ARS, ERRC, Wyndmoor, PA, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 565. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Apr 2002
Last Updated on STN: 10 Apr 2002

AB Single nucleotide **mutations** in the promoter of the csgD gene of Escherichia coli O157:H7 strains 43895 and 43894 result in a curli-expressing and a Congo red dye-**binding** phenotype which can revert to a non-curliated phase under the appropriate growth conditions. The white variants of 43895 and 43894 were no more invasive for cultured HEp-2 cells than a non-invasive, E. coli control. However, the red variants of both strains were significantly more invasive than the white variants and showed no statistical difference in invasiveness than an enteroinvasive E. coli control. Both red and white variants of 43895 were able to colonize the gastrointestinal tract of mice in a streptomycin-treated mouse model. However, the survival of mice orally challenged with the red variant was significantly shorter than that of mice receiving the white variant. Red and white variants of strains 43894 and 43895 were compared for **Shiga-like toxin** (SLT) production. The 50% cytotoxic dose (CD50) for both secreted and cell-associated SLT, determined for the red variants of each strain, were not different from the CD50 of the counterpart white variants. This result indicates that the differences in mouse mortality were not the result of differences in SLT production. The results of this study suggest that the Congo red dye-binding variants of strains 43895 and 43894, which contain promoter alterations allowing for greater expression from csgD, display important functional differences compared to the white variants.

L6 ANSWER 14 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1996:259474 BIOSIS
DOCUMENT NUMBER: PREV199698815603
TITLE: Determination of a second **binding** site in the **Shiga-like toxin** family **binding** subunits by **mutational** analysis.
AUTHOR(S): Banerjee, L. [Reprint author]; Agha, R.; Lingwood, C. A.; Brunton, J.
CORPORATE SOURCE: Samuel Lunenfeld Res. Inst., Mt. Sinai Hosp., Toronto, ON, Canada
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1996) Vol. 96, No. 0, pp. 189.
Meeting Info.: 96th General Meeting of the American Society for Microbiology. New Orleans, Louisiana, USA. May 19-23, 1996.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 31 May 1996
Last Updated on STN: 11 Jul 1996

L6 ANSWER 15 OF 35 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2003202412 EMBASE
TITLE: Effects of HIV-1 Nef on retrograde transport from the

plasma membrane to the endoplasmic reticulum.
AUTHOR: Johannes L.; Pezo V.; Mallard F.; Tenza D.; Wiltz A.; Saint-Pol A.; Helft J.; Antony C.; Benaroch P.
CORPORATE SOURCE: L. Johannes, CNRS UMR144, Institut Curie, 26 rue d'Ulm, F-75248 Paris Cedex 05, France. Ludger.Johannes@curie.fr
SOURCE: Traffic, (1 May 2003) 4/5 (323-332).
Refs: 42
ISSN: 1398-9219 CODEN: TRAFFA
COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB HIV-1 Nef protein down-regulates several important immunoreceptors through interactions with components of the intracellular sorting machinery. Nef expression is also known to induce modifications of the endocytic pathway. Here, we analyzed the effects of Nef on retrograde transport, from the plasma membrane to the endoplasmic reticulum using **Shiga toxin B**-subunit (STxB). Nef expression inhibited access of STxB to the endoplasmic reticulum, but did not modify the surface expression level of STxB receptor, Gb(3), nor its internalization rate as measured with a newly developed assay. **Mutation** of the myristoylation site or of a di-leucine motif of Nef involved in the interaction with the clathrin adaptor complexes AP1 and AP2 abolished the inhibition of retrograde transport. In contrast, **mutations** of Nef motifs known to interact with PACS-1, βCOP or a subunit of the v-ATPase did not modify the inhibitory activity of Nef on retrograde transport. Ultrastructural analysis revealed that Nef was present in clusters located on endosomal or Golgi membranes together with internalized STxB. Furthermore, in strongly Nef-expressing cells, STxB accumulated in endosomal structures that labeled with AP1. Our observations show that Nef perturbs retrograde transport between the early endosome and the endoplasmic reticulum. The potential transport steps targeted by Nef are discussed.

L6 ANSWER 16 OF 35 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2003108177 EMBASE
TITLE: Mutations in hns reduce the adherence of **Shiga toxin**-producing E. coli 091:H21 strain B2F1 to human colonic epithelial cells and increase the production of hemolysin.
AUTHOR: Scott M.E.; Melton-Celsa A.R.; O'Brien A.D.
CORPORATE SOURCE: A.D. O'Brien, Dept. of Microbiology and Immunology, F. Edward Hebert Sch. of Med., Uniformed Serv. Univ. the Hlth. Sci., 4301 Jones Bridge Road, Bethesda, MD 20814-4799, United States. aobrien@usuhs.mil
SOURCE: Microbial Pathogenesis, (1 Mar 2003) 34/3 (155-159).
Refs: 32
ISSN: 0882-4010 CODEN: MIPAEV
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
005 General Pathology and Pathological Anatomy
025 Hematology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Shiga toxin**-producing Escherichia coli (STEC) 091:H21 strain B2F1, an isolate from a patient with the hemolytic uremic syndrome (HUS), produces elastase-activatable **Shiga toxin** (Stx) type 2d and adheres well to human colonic epithelial T84 cells. This adherence phenotype occurs even though B2F1 does not contain the locus of

enterocyte effacement (LEE) that encodes the primary adhesin for E. coli O157:H7. To attempt to identify genes involved in **binding** of B2F1 to T84 cells a bank of mini-Tn5phoACm(r) transposon mutants of this strain was generated. Several of these mutants exhibited a reduced adherence phenotype, but none of the insertions in these mutants were within putative adhesin genes. Rather, insertional **mutations** within hns resulted in the loss of adherence. Moreover, the hns mutant also displayed an increase in the production of hemolysin and alkaline phosphatase and a loss of motility with no change in Stx2d-activatable expression levels. When B2F1 was cured of the large plasmid that encodes the hemolysin, the resulting strain adhered well to T84 cells. However, an hns mutant of the plasmid-cured B2F1 strain exhibited a reduction in adherence to T84 cells. Taken together, these results indicate that H-NS regulates the expression of several genes and some potential virulence factors in the intimin-negative B2F1 STEC strain and that the large plasmid is not required for T84 cell colonization.

L6 ANSWER 17 OF 35 EMBASE
on STN

ACCESSION NUMBER: 2002267215 EMBASE
TITLE: Enterohemolysin operon of **Shiga toxin** -producing Escherichia coli: A virulence function of inflammatory cytokine production from human monocytes.
AUTHOR: Taneike I.; Zhang H.-M.; Wakisaka-Saito N.; Yamamoto T.
CORPORATE SOURCE: T. Yamamoto, Division of Bacteriology, Dept. of Infectious Disease Control, N. Univ. Grad. Sch. Med./Dental Sci., 757 Ichibanchou, Asahimachidori, Niigata, Japan. tatsuoy@med.niigata-u.ac.jp
SOURCE: FEBS Letters, (31 Jul 2002) 524/1-3 (219-224). Refs: 44
ISSN: 0014-5793 CODEN: FEBLAL
PUBLISHER IDENT.: S 0014-5793(02)03027-2
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Shiga toxin**-producing Escherichia coli (STEC) is associated with hemolytic uremic syndrome (HUS). Although most clinical isolates of STEC produce hemolysin (called enterohemolysin), the precise role of enterohemolysin in the pathogenesis of STEC infections is unknown. Here we demonstrated that E. coli carrying the cloned enterohemolysin operon (hlyC, A, **B**, D genes) from an STEC human strain induced the production of interleukin-1β (IL-1β) through its mRNA expression but not tumor necrosis factor-α from human monocytes. No IL-1β release was observed with an enterohemolysin (HlyA)-negative, isogenic E. coli strain carrying a **mutation** in the hlyA gene. The data suggest that enterohemolysin, a pore-forming **toxin**, induces the production of IL-1β, which is one of serum risk markers for HUS. .COPYRGT. 2002 Federation of European Biochemical Societies. Published by Elsevier Science **B**.V. All rights reserved.

L6 ANSWER 18 OF 35 EMBASE
on STN

ACCESSION NUMBER: 2001258702 EMBASE
TITLE: Differences in levels of secreted locus of enterocyte effacement proteins between human disease-associated and bovine Escherichia coli O157.
AUTHOR: McNally A.; Roe A.J.; Simpson S.; Thomson-Carter F.M.; Elaine Hoey D.E.; Currie C.; Chakraborty T.; Smith D.G.E.; Gally D.L.
CORPORATE SOURCE: D.L. Gally, ZAP Laboratory, Department of Veterinary

Pathology, Teviot Place, Edinburgh EH8 9AG, United Kingdom.
dgally@ed.ac.uk
SOURCE: Infection and Immunity, (2001) 69/8 (5107-5114).
Refs: 45
ISSN: 0019-9567 CODEN: INFIBR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
005 General Pathology and Pathological Anatomy
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Ongoing extensive epidemiological studies of verotoxin-carrying Escherichia coli O157 (stx(+) eae(+)) have shown this bacterial pathogen to be common in cattle herds in the United States and the United Kingdom. However, the incidence of disease in humans due to this pathogen is still very low. This study set out to investigate if there is a difference between strains isolated from human disease cases and those isolated from asymptomatic cattle which would account for the low disease incidence of such a ubiquitous organism. The work presented here has compared human disease strains from both sporadic and outbreak cases with a cross-section, as defined by pulsed-field gel electrophoresis, of E. coli O157 strains from cattle. Human (n = 22) and bovine (n = 31) strains were genotyped for carriage of the genes for **Shiga-like toxin** types 1, 2, and 2c; E. coli secreted protein genes espA, espB, and espP; the enterohemolysin gene; eae (intimin); ast (enteroaggregative E. coli stable **toxin** [EAST]); and genes for common E. coli adhesins. Strains were also phenotyped for hemolysin, EspP, Tir, and EspD expression as well as production of actin and cytoskeletal rearrangement associated with attaching and effacing (A/E) lesions on HeLa cells. The genotyping confirmed that there was little difference between the two groups, including carriage of stx(2) and stx(2c), which was similar in both sets. ast alleles were confirmed to all contain **mutations** that would prevent EAST expression. espP **mutations** were found only in cattle strains (5 of 30). Clear differences were observed in the expression of locus of enterocyte effacement (LEE)-encoded factors between strains and in different media. EspD, as an indicator of LEE4 (espA, -**B**, and -D) expression, and Tir levels in supernatants were measured. Virtually all strains from both sources could produce EspD in Luria-Bertani broth, although at very different levels. Standard trichloroacetic acid precipitation of secreted proteins from tissue culture medium produced detectable levels of EspD from the majority of strains of human origin (15 of 20) compared with only a few (4 of 20) bovine strains ($P < 0.001$), which is indicative of much higher levels of protein secretion from the human strains. Addition of bovine serum albumin carrier protein before precipitation and enhanced detection techniques confirmed that EspD could be detected after growth in tissue culture medium for all strains, but levels from strains of human origin were on average 90-fold higher than those from strains of bovine origin. In general, levels of secretion also correlated with ability to form A/E lesions on HeLa cells, with only the high-level protein secretors in tissue culture medium exhibiting a localized adherence phenotype. This research shows significant differences between human- and bovine-derived E. coli O157 (stx(+) eae(+)) strains and their production of certain LEE-encoded virulence factors. These data support the recent finding of Kim et al. (J. Kim, J. Nietfeldt, and A. K. Benson, Proc. Natl. Acad. Sci. USA 96:13288-13293, 1999) proposing different E. coli O157 lineages in cattle and humans and extend the differential to the regulation of virulence factors. Potentially only a subset of E. coli O157 isolates (stx(+) eae(+)) in cattle may be capable of causing severe disease in humans.

on STN
ACCESSION NUMBER: 2000353423 EMBASE
TITLE: Production of **Shiga toxin** by Escherichia coli measured with reference to the membrane vesicle-associated toxins.
AUTHOR: Yokoyama K.; Horii T.; Yamashino T.; Hashikawa S.; Barua S.; Hasegawa T.; Watanabe H.; Ohta M.
CORPORATE SOURCE: M. Ohta, Department Molecular Bacteriology, Nagoya University, Postgraduate School of Medicine, 65 Tsurumaicho, Showa, Nagoya, Aichi 466-8550, Japan. mohta@tsuru.med.nagoya-u.ac.jp
SOURCE: FEMS Microbiology Letters, (1 Nov 2000) 192/1 (139-144). Refs: 25
ISSN: 0378-1097 CODEN: FMLED7
PUBLISHER IDENT.: S 0378-1097(00)00424-9
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Production of **Shiga toxin** (Stx) 1 and 2 from Stx-producing Escherichia coli (STEC) was measured with reference to the membrane vesicle (MV)-associated toxins. An immunoblot analysis method using specific antibodies for Stx1 and Stx2 was developed for the detection of the extracellular toxins. All 46 STEC isolates, studied including 30 O157 and 16 other O-antigenic isolates, released Stx1 and Stx2 as MV-associated and MV-removed fractions under aerobic and anaerobic conditions. Treatment of vesicles with polymyxin **B** that disrupted MVs increased the release of Stx1 and Stx2. Therefore, delivery of Stx1 and Stx2 by MVs is a general mechanism in STEC. Stx1 remained within MVs rather than in the MV-removed fraction under an aerobic culture condition. On the other hand, a larger proportion of Stx2 was detected in the MV-removed fraction. The kinetic patterns of the release of the toxins from STEC strains showed that both Stx1 and Stx2 were released into the growth medium during the exponential growth phase. An rpoS-deficient **mutation** did not have altered levels of extracellular Stx1 and Stx2, supporting the idea that Stx1 and Stx2 are produced during exponential growth phase. Copyright (C) 2000 Federation of European Microbiological Societies.

L6 ANSWER 20 OF 35 EMBASE
on STN
ACCESSION NUMBER: 2000099478 EMBASE
TITLE: A mutant **Shiga-like toxin** lie bound to its receptor Gb3: Structure of a group II **Shiga-like toxin** with altered binding specificity.
AUTHOR: Ling H.; Pannu N.S.; Boodhoo A.; Armstrong G.D.; Clark C.G.; Brunton J.L.; Read R.J.
CORPORATE SOURCE: R.J. Read, Department of Biochemistry, University of Alberta, Edmonton, Alta. T6G 2H7, Canada. rjr27@cam.ac.uk
SOURCE: Structure, (15 Mar 2000) 8/3 (253-264). Refs: 72
ISSN: 0969-2126 CODEN: STRUE6
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Background: **Shiga-like** toxins (SLTs) are produced by the pathogenic strains of Escherichia coli that cause hemorrhagic colitis and hemolytic uremic syndrome. These diseases in humans are generally

associated with group II family members (SLT-II and SLT-IIc), whereas SLT-Ile (pig edema **toxin**) is central to edema disease of swine. The pentameric **B**-subunit component of the majority of family members binds to the cell-surface glycolipid globotriaosyl ceramide (Gb3), but globotetraosyl ceramide (Gb4) is the preferred receptor for SLT-Ile. A double-mutant of the SLT-Ile **B** subunit that reverses two sequence differences from SLT-II (GT3; Gln65→Glu, Lys67→Gln, SLT-I numbering) has been shown to bind more strongly to Gb3 than to Gb4. Results: To understand the molecular basis of receptor **binding** and specificity, we have determined the structure of the GT3 mutant **B** pentamer, both in complex with a Gb3 analogue (2.0 Å resolution; R = 0.155, R(free) = 0.194) and in its native form (2.35 Å resolution; R = 0.187, R(free) = 0.232). Conclusions: These are the first structures of a member of the medically important group II **Shiga -like** toxins to be reported. The structures confirm the previous observation of multiple **binding** sites on each SLT monomer, although **binding** site 3 is not occupied in the GT3 structure. Analysis of the **binding** properties of mutants suggests that site 3 is a secondary Gb4-**binding** site. The two **mutated** residues are located appropriately to interact with the extra βGalNAc residue on Gb4. Differences in the **binding** sites provide a molecular basis for understanding the tissue specificities and pathogenic mechanisms of members of the SLT family.

L6 ANSWER 21 OF 35 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 1999175485 EMBASE
TITLE: Proteolytic cleavage of the A subunit is essential for maximal cytotoxicity of Escherichia coli O157:H7 **Shiga-like toxin**-1.
AUTHOR: Lea N.; Lord J.M.; Roberts L.M.
CORPORATE SOURCE: J.M. Lord, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom. ml@dna.bio.warwick.ac.uk
SOURCE: Microbiology, (1999) 145/5 (999-1004).
Refs: 25
ISSN: 1350-0872 CODEN: MROBEO
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Members of the bacterial **Shiga toxin** family consist of a single A subunit that is non-covalently associated with a pentamer of **B** subunits. These toxins bind to receptors on susceptible mammalian cells and enter the cells by endocytic uptake. During cell entry, the 32 kDa A subunit is cleaved by the membrane-anchored protease furin to generate a catalytically active, 27.5 kDa A1 fragment and a 4.5 kDa A2 fragment. Previous studies have shown that **mutating** the furin site to prevent cleavage did not significantly affect **toxin** potency, suggesting that cleavage is not required for **toxin** activity. Here it is confirmed that preventing cleavage at the usual processing site does not prevent proteolytic processing of the Escherichia coli **Shiga-like toxin**-1 A subunit. However, simultaneous **mutation** of both the primary furin-recognition site and a nearby putative furin cleavage site did prevent intracellular processing of the A subunit. Comparison of the cytotoxicities of purified recombinant toxins to cultured mammalian cells demonstrated that even on prolonged incubation with **toxin**, the unprocessed mutant was 60-fold less toxic than the wild-type protein or other mutants still capable of being proteolytically processed during cell entry.

L6 ANSWER 22 OF 35 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 1998295686 EMBASE

TITLE: Identification and characterization of a newly isolated **shiga toxin** 2- converting phage from **shiga toxin**-producing Escherichia coli.

AUTHOR: Watarai M.; Sato T.; Kobayashi M.; Shimizu T.; Yamasaki S.; Tobe T.; Sasakawa C.; Takeda Y.

CORPORATE SOURCE: Y. Takeda, Research Institute, International Med. Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162, Japan. resedr@imcj.go.jp

SOURCE: Infection and Immunity, (1998) 66/9 (4100-4107).
Refs: 59
ISSN: 0019-9567 CODEN: INFIBR

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB **Shiga** toxins 1 (Stx1) and 2 (Stx2) are encoded by **toxin** -converting bacteriophages of Stx-producing Escherichia coli (STEC), and so far two Stx1- and one Stx2-converting phages have been isolated from two STEC strains (A.D. O'Brien, J. W. Newlands, S. F. Miller, R. K. Holmes, H. W. Smith, and S. **B**. Formal, Science 226: 694-696, 1984). In this study, we isolated two Stx2-converting phages, designated Stx2Φ-I and Stx2Φ-II, from two clinical strains of STEC associated with the outbreaks in Japan in 1996 and found that Stx2Φ-I resembled 933W, the previously reported Stx2-converting phage, in its infective properties for E. coli K-12 strain C600 while Stx2Φ-II was distinct from them. The sizes of the plaques of Stx2Φ-I and Stx2Φ-II in C600 were different; the former was larger than the latter. The restriction maps of Stx2Φ-I and Stx2Φ-II were not identical; rather, Stx2Φ-II DNA was approximately 3 kb larger than Stx2Φ-I DNA. Furthermore, Stx2Φ-I and Stx2Φ-II showed different phage immunity, with Stx2Φ-I and 933W belonging to the same group. Infection of C600 by Stx2Φ-I or 933W was affected by environmental osmolarity differently from that by Stx2Φ-II. When C600 was grown under conditions of high osmolarity, the infectivity of Stx2Φ-I and 933W was greatly decreased compared with that of Stx2Φ-II. Examination of the plating efficiency of the three phages for the defined **mutations** in C600 revealed that the efficiency of Stx2Φ-I and 933W for the fadL mutant decreased to less than 10-7 compared with that for C600 whereas the efficiency of Stx2Φ-II decreased to 0.1% of that for C600. In contrast, while the plating efficiency of Stx2Φ-II for the lamb mutant decreased to a low level (0.05% of that for C600), the efficiencies of Stx2Φ-I and 933W were not changed. This was confirmed by the phage neutralization experiments with isolated outer membrane fractions from C600, fadL mutant, or lamB mutant or the purified His6-tagged FadL and Lamb proteins. Based on the data, we concluded that FadL acts as the receptor for Stx2Φ-I and Stx2Φ-II whereas LamB acts as the receptor only for Stx2Φ-II.

L6 ANSWER 23 OF 35 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 97129503 EMBASE

DOCUMENT NUMBER: 1997129503

TITLE: Role of the disulfide bond in **shiga toxin** A-chain for **toxin** entry into cells.

AUTHOR: Garred O.; Dubinina E.; Polesskaya A.; Olsnes S.; Kozlov J.; Sandvig K.

CORPORATE SOURCE: K. Sandvig, Institute for Cancer Research, Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway

SOURCE: Journal of Biological Chemistry, (1997) 272/17 (11414-11419).
Refs: 36
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Shiga toxin** consists of an enzymatically active A-chain and a pentameric **binding** subunit. The A-chain has a trypsin-sensitive region, and upon cleavage two disulfide bonded fragments, A1 and A2, are generated. To study the role of the disulfide bond, it was eliminated by **mutating** cysteine 242 to serine. In T47D cells this **mutated toxin** was more toxic than wild type **toxin** after a short incubation, whereas after longer incubation times wild type **toxin** was most toxic. Cells cleaved not only wild type but also **mutated** A- chain into A1 and A2 fragments. The **mutated** A-chain was more sensitive than wild type **toxin** to Pronase, and it was degraded at a higher rate in T47D cells. Subcellular fractionation demonstrated transport of both wild type and **mutated toxin** to the Golgi apparatus. Brefeldin A, which disrupts the Golgi apparatus, protected not only against **Shiga toxin** but also against the **mutated toxin**, indicating involvement of the Golgi apparatus. After prebinding of **Shiga**(C242S) **toxin** to wells coated with the **Shiga toxin** receptor, Gb3, trypsin treatment induced dissociation of A1 from the **toxin**- receptor complex demonstrating that in addition to stabilizing the A-chain, the disulfide bond prevents dissociation of the A1 fragment from the **toxin**- receptor complex.

L6 ANSWER 24 OF 35 EMBASE
on STN
ACCESSION NUMBER: 97052832 EMBASE
DOCUMENT NUMBER: 1997052832
TITLE: Investigation of ribosome binding by the **shiga toxin** A1 subunit, using competition and site-directed mutagenesis.
AUTHOR: Skinner L.M.; Jackson M.P.
CORPORATE SOURCE: M.P. Jackson, Wayne State Univ. School of Medicine, 540 E. Canfield Ave., Detroit, MI 48201, United States. mpjacks@med.wayne.edu
SOURCE: Journal of Bacteriology, (1997) 179/4 (1368-1374).
Refs: 34
ISSN: 0021-9193 CODEN: JOBAAY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The enzymatic subunit of **Shiga toxin** (StxA1) is a member of the ribosome-inactivating protein (RIP) family, which includes the ricin A chain as well as other examples of plant toxins. StxA1 catalytically depurinates a well-conserved GAGA tetra-loop of 28S rRNA which lies in the acceptor site of eukaryotic ribosomes. The specific activities of native StxA1, as well as **mutated** forms of the enzyme with substitutions in catalytic site residues, were measured by an in vitro translation assay. Electroporation was developed as an alternative method for the delivery of purified A1 polypeptides into Vero cells. Site-directed mutagenesis coupled with N-bromosuccinimide

modification indicated that the sole tryptophan residue of StxA1 is required for **binding** it to the 28S rRNA backbone. Northern analysis established that the catalytic site substitutions reduced enzymatic activity by specifically interfering with the capacity of StxA1 to depurinate 28S rRNA. Ribosomes were protected from StxA1 by molar excesses of tRNA and free adenine, indicating that RIPs have the capacity to enter the acceptor site groove prior to **binding** and depurinating the GAGA tetra-loop.

L6 ANSWER 25 OF 35 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 93311219 EMBASE
DOCUMENT NUMBER: 1993311219
TITLE: Proteolytic cleavage at arginine residues within the hydrophilic disulphide loop of the Escherichia coli **Shiga-like toxin** I A subunit is not essential for cytotoxicity.
AUTHOR: Burgess B.J.; Roberts L.M.
CORPORATE SOURCE: Department of Biological Sciences, University of Warwick,Coventry CV4 7AL, United Kingdom
SOURCE: Molecular Microbiology, (1993) 10/1 (171-179).
ISSN: 0950-382X CODEN: MOMIEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Escherichia coli **Shiga-like toxin** I is a type II ribosome-inactivating protein composed of an A subunit with RNA-specific N-glycosidase activity, non-covalently associated with a pentamer of **B** subunits possessing affinity for galabiose-containing glycolipids. The A subunit contains a single intrachain disulphide bond encompassing a hydrophilic sequence containing two trypsin-sensitive arginine residues. By analogy with other bacterial toxins it has been proposed that proteolytic nicking, deemed essential for a cytotoxic effect, occurs within this disulphide-bonded loop to generate the A1 and A2 fragments. Reduced A1 is then believed to translocate an internal membrane to inactivate protein synthesis in the cytosol. In this report, the disulphide-loop arginines of the SLT I A subunit were **mutated** to block the specific proteolysis presumed to occur. However, the mutant generated remained an effective **toxin** having similar catalytic activity to wild-type **toxin** and only a marginally reduced cytotoxicity towards cultured cells. We conclude that the disulphide-loop arginine residues are not the unique and essential processing sites previously assumed, but that processing may occur at alternative accessible sites to compensate for loss of target sites within the loop.

L6 ANSWER 26 OF 35 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 92314587 EMBASE
DOCUMENT NUMBER: 1992314587
TITLE: Identification of a B subunit gene promoter in the **Shiga toxin** operon of Shigella dysenteriae 1.
AUTHOR: Habib N.F.; Jackson M.P.
CORPORATE SOURCE: Immunology/Microbiology Department, Wayne State Univ. School of Medicine, 540 East Canfield Avenue,Detroit, MI 48201, United States
SOURCE: Journal of Bacteriology, (1992) 174/20 (6498-6507).
ISSN: 0021-9193 CODEN: JOBAAY
COUNTRY: United States

DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The **Shiga toxin** operon (stx) is composed of A and **B** subunit genes which are transcribed as a bicistronic mRNA from a promoter which lies 5' to the stxA gene. Northern (RNA) blot and primer extension analyses revealed the existence of a second stxB gene transcript. Recombinant plasmids which carried the stxB gene without the stx operon promoter and with the influence of a vector promoter abrogated produced STX **B** polypeptides, suggesting that the stxB gene mRNA was transcribed from an independent promoter and was not produced by endoribonucleolytic processing of the bicistronic mRNA. Examination of the DNA sequences 5' to the stxB gene transcription initiation site which were carried by the recombinant plasmids revealed a region with high homology to the consensus for Escherichia coli promoters. Deletion and **mutation** of this region affected StxB and holotoxin production, establishing its role in the regulation of the stxB gene. Comparison of the promoters by using a transcription analysis vector revealed that the stxB gene promoter differed from the stx operon promoter in that was approximately sixfold less efficient and was not repressed by iron. Identification of a second promoter in the stx operon indicates that independent transcription of the stxB gene may regulate overproduction of the STX **B** polypeptides and may contribute to the 1A:5B subunit stoichiometry of the holotoxin.

L6 ANSWER 27 OF 35 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 88052235 EMBASE
DOCUMENT NUMBER: 1988052235
TITLE: Iron regulation of **Shiga-like toxin** expression of Escherichia coli is mediated by the fur locus.
AUTHOR: Calderwood S.B.; Mekalanos J.J.
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, United States
SOURCE: Journal of Bacteriology, (1987) 169/10 (4759-4764). ISSN: 0021-9193 CODEN: JOBAAY
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Shiga-like toxin** is an iron-regulated cytotoxin quite similar to **Shiga toxin** from Shigella dysenteriae 1. The structural genes for **Shiga-like toxin** in Escherichia coli (sltA and sltB) appear to be transcribed as an operon from a promoter upstream of sltA. We used a gene fusion between the promoter and proximal portion of sltA with the gene for bacterial alkaline phosphatase to assess the regulation of **toxin** expression. Growth in low-iron conditions resulted in a 13- to 16-fold increase in alkaline phosphatase activity. In the presence of a null **mutation** in the fur locus, however, alkaline phosphatase activity was constitutively high regardless of the iron concentration. These data indicate negative regulation of the slt operon by the fur gene product. We used deletion analysis of the region upstream of the gene fusion to localize the promoter of the slt operon and to show that a region of DNA between the -35 and -10 boxes is necessary for iron regulation of slt expression. In this region, there is a 21-base-pair dyad repeat that is homologous to similar dyads in the promoter regions of three other fur-regulated genes. This region of dyad symmetry may represent an operator **binding** site for the Fur protein in the presence of

iron.

L6 ANSWER 28 OF 35 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:713165 CAPLUS
DOCUMENT NUMBER: 135:256122
TITLE: Chimeric nontoxic mutants of enterotoxins as mucosal adjuvants for cell-mediated or humoral immunity
INVENTOR(S): McGhee, Jerry; Kiyono, Hiroshi; Takeda, Yoshifumi; Ohmura, Mari; Yamamoto, Shingo
PATENT ASSIGNEE(S): Uab Research Foundation, USA
SOURCE: PCT Int. Appl., 23 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001070257	A1	20010927	WO 2001-US8582	20010316
W: AU, CA, GB, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
US 2002142006	A1	20021003	US 2001-809033	20010316
EP 1272210	A1	20030108	EP 2001-920475	20010316
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY, TR				
PRIORITY APPLN. INFO.:			US 2000-190058P	P 20000317
			WO 2001-US8582	W 20010316

AB Customized chimeric mutants having a **mutated** A chain from a first toxin and a **B** chain from a second toxin provide customized constructs which can be directed to selectively provide cell-mediated immune response or humoral immune response. The first enterotoxin is **mutated** A subunit of cholera toxin and the second enterotoxin is a non-**mutated B** chain of labile toxin of Escherichia coli.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 29 OF 35 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:283986 CAPLUS
DOCUMENT NUMBER: 134:309693
TITLE: AB5 toxin B subunit mutants with altered chemical conjugation characteristics
INVENTOR(S): Handley, Harold H.; Haaparanta, Tapio; Ewalt, Karla L.
PATENT ASSIGNEE(S): Active Biotech AB, Swed.
SOURCE: PCT Int. Appl., 77 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001027144	A2	20010419	WO 2000-US27607	20001005
WO 2001027144	A3	20020117		
W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM,				

TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1222202	A2	20020717	EP 2000-968795		20001005
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL					
JP 2003511061	T2	20030325	JP 2001-530362		20001005
NZ 518342	A	20040430	NZ 2000-518342		20001005
PRIORITY APPLN. INFO.:			US 1999-158561P	P	19991008
			WO 2000-US27607	W	20001005

AB A recombinant AB5 **B** subunit protein including at least one **mutation**, wherein the **mutation** alters the number of amino acid residues available for chemical modification as compared to a wild type AB5 **B** subunit protein, and wherein said recombinant protein retains an effective target ligand bind affinity. For example, specifically designed **mutations** are produced in the cholera Toxin **B** subunit (CTB) such that it can still bind with high affinity to its receptor, Gm-1, but can be specifically covalently linked at lysines or cysteines to an immunogen or vaccine. The vaccine produced from this coupling is a mucosal vaccine which has high immunogenicity due to the interaction with the CTB. The vaccine can be produced inexpensively and easily. Alternatively, a technique is disclosed for treating CTB such that non-covalent coupling to a vaccine or immunogen can occur. The disclosed CTB can not only be used as vaccine but also as bioactive mol. delivery agent.

L6 ANSWER 30 OF 35 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER:	2001:228924 CAPLUS
DOCUMENT NUMBER:	134:265140
TITLE:	Mutated anthrax toxin protective antigen proteins that specifically target cells containing high amounts of cell-surface metalloproteinases or plasminogen activator receptors
INVENTOR(S):	Leppla, Stephen H.; Liu, Shi-Hui; Netzel-Arnett, Sarah; Hansen-Birkedal, Henning; Bugge, Thomas
PATENT ASSIGNEE(S):	Government of the United States of America, as Represented by the Secretary, Department of Health and Human Services, USA
SOURCE:	PCT Int. Appl., 77 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	1
PATENT INFORMATION:	

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001021656	A2	20010329	WO 2000-US26192	20000922
WO 2001021656	A3	20020117		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2001025725	A5	20010424	AU 2001-25725	20000922
AU 771632	B2	20040401		

EP 1214340 A2 20020619 EP 2000-989184 20000922
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL
PRIORITY APPLN. INFO.: US 1999-155961P P 19990924
WO 2000-US26192 W 20000922

AB The present invention provides methods of specifically targeting compds. to cells overexpressing matrix metalloproteinases, plasminogen activators, or plasminogen activator receptors, by administering a compound and a mutant protective antigen protein comprising a matrix metalloproteinase or a plasminogen activator-recognized cleavage site in place of the native protective antigen furin-recognized cleavage site, wherein the mutant protective antigen is cleaved by a matrix metalloproteinase or a plasminogen activator overexpressed by the cell, thereby translocating into the cell a compound comprising a lethal factor polypeptide comprising a protective antigen binding site.

L6 ANSWER 31 OF 35 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:17137 CAPLUS
DOCUMENT NUMBER: 118:17137
TITLE: Construction of stable LamB-**Shiga toxin** B subunit hybrids: analysis of expression in Salmonella typhimurium aroA strains and stimulation of B subunit-specific mucosal and serum antibody responses
AUTHOR(S): Su, Guo Fu; Brahmbhatt, Himanshu N.; Wehland, Juergen; Rohde, Manfred; Timmis, Kenneth N.
CORPORATE SOURCE: Dep. Microbiol., Natl. Res. Cent. Biotechnol., Braunschweig, Germany
SOURCE: Infection and Immunity (1992), 60(8), 3345-59
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The complete **Shiga toxin** B subunit and two N-terminal segments of the B subunit have been inserted into a cell surface-exposed loop of the LamB protein, and expression of the hybrid proteins from three different promoter systems, i.e., (i) an in vitro-inducible tac promoter that provides high-level expression, (ii) the iron-regulated aerobactin promoter presumably induced in vivo under the iron-limiting conditions of the intestinal mucosal environment, and (iii) a synthetic, modified β-lactamase promoter providing moderate level constitutive expression, has been analyzed in Escherichia coli, Salmonella typhimurium, and attenuated antigen carrier strains of S. typhimurium (aroA mutants). The hybrid vaccine strains were used to immunize mice by the oral and i.p. routes. S. typhimurium aroA mutants apparently have a membrane export defect which prevents the transport of LamB and its derivs. across the cytoplasmic membrane. High-level expression of hybrid proteins through use of the tac promoter proved deleterious to the vaccine strains and prevented the production of viable cells at reasonable cell densities. The lower levels of gene expression observed with the β-lactamase and aerobactin promoters did not have this effect. Immunization of mice with S. typhimurium aroA strains carrying the hybrid genes expressed from these two promoters resulted in significant B subunit-specific mucosal and serum antibody responses. This suggests that such expression systems may be useful when incorporated into candidate antidysentery live oral vaccines for inducing protection against the effect of **Shiga toxin** in infections caused by Shigella dysenteriae 1 and other **Shiga toxin**- or **Shiga-like toxin**-producing pathogens.

L6 ANSWER 32 OF 35 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 2002-690113 [74] WPIDS
CROSS REFERENCE: 2000-532863 [48]; 2002-557291 [59]; 2002-598710 [64];

2002-635659 [68]; 2002-635674 [68]
DOC. NO. CPI: C2002-195008
TITLE: Immunogenic composition, useful to prevent or treat pathogenic bacterial infection, comprises live bacteria with DNA adenine methylase activity altered relative to wild-type, and which also express a heterologous antigen.
DERWENT CLASS: B04 D13 D16
INVENTOR(S): HEITHOFF, D M; LOW, D A; MAHAN, M J; SINSHEIMER, R L
PATENT ASSIGNEE(S): (HEIT-I) HEITHOFF D M; (LOWD-I) LOW D A; (MAHA-I) MAHAN M J; (SINS-I) SINSHEIMER R L
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2002081317	A1	20020627	(200274)*		44

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
US 2002081317	A1	Provisional	US 1999-183043P	19990202
		Provisional	US 1999-198250P	19990505
		CIP of	US 2000-495614	20000201
		CIP of	US 2000-612116	20000707
			US 2001-927788	20010809

PRIORITY APPLN. INFO: US 2001-927788 20010809; US 1999-183043P 19990202; US 1999-198250P 19990505; US 2000-495614 20000201; US 2000-612116 20000707
AN 2002-690113 [74] WPIDS
CR 2000-532863 [48]; 2002-557291 [59]; 2002-598710 [64]; 2002-635659 [68]; 2002-635674 [68]
AB US2002081317 A UPAB: 20021118
NOVELTY - An immunogenic composition (I) comprises a live bacteria with DNA adenine methylase (Dam) activity altered relative to that of the wild-type, where the alteration renders the bacteria attenuated, and a first heterologous nucleotide sequence operatively inserted in the bacteria, where the sequence expresses a heterologous antigen.

ACTIVITY - Antibacterial; Antiparasitic; Fungicide; Protozoacide; Virucide; Tuberculostatic; Immunostimulant.

No supporting data.

MECHANISM OF ACTION - Vaccine.

The ability of Dam- and Dam overproducing Salmonella to elicit cross-protection was tested. BALB/c mice were immunized with 1 multiply 109 Dam- or Dam overproducing Salmonella administered orally. Mice were challenged with the virulent Salmonella serotype eleven weeks post-immunization, which was six weeks after the vaccine strains were cleared from murine tissues, including Peyer's patches, mesenteric lymph nodes, liver, and spleen. The results showed that mice were protected against a heterologous challenge eleven weeks post immunization. Immunization with Dam- S.enteritidis conferred cross-protection against challenge with 109 S.typhimurium and 109 S.dublin after five weeks and conferred cross-protection for even longer periods. One third of mice vaccinated with a single oral dose of Dam S.enteritidis survived a virulent heterologous challenge eleven weeks post-immunization of 104 above the lethal dose required to kill 50% of the animals against strains S.dublin and S.typhimurium, comparable to the level of survival observed upon homologous challenge. To test whether Dam overproducing strains

elicited protective immune responses to homologous and heterologous Salmonella serotypes similar to Dam strains, mice were immunized with Dam-overproducing S.typhimurium. 75% of immunized mice survived a challenge dose of 1000-fold above the LD50 of S.dublin and S.typhimurium. Taken together, these studies indicated that Salmonella strains that under- or over-produced Dam were highly attenuated and served as protective live vaccines against homologous and at least some heterologous serotypes.

USE - (I) is useful for eliciting an immune response in an individual, and for treating or preventing pathogenic bacterial, viral, fungal, parasitic and vector borne infections (all claimed), especially Salmonella infections.

Dwg.0/9

L6 ANSWER 33 OF 35 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-431269 [37] WPIDS
DOC. NO. CPI: C2000-131046
TITLE: Protein transduction system for treating cancer and pathogenic infections has a fusion protein comprising a protein transduction domain covalently linked to a cytotoxic domain.
DERWENT CLASS: B04 D16
INVENTOR(S): DOWDY, S F
PATENT ASSIGNEE(S): (UNIW) UNIV WASHINGTON
COUNTRY COUNT: 87
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000034308	A2	20000615	(200037)*	EN	127
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW					
AU 2000021728	A	20000626	(200045)		
EP 1137664	A2	20011004	(200158)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
JP 2002531113	W	20020924	(200278)		173

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000034308	A2	WO 1999-US29289	19991210
AU 2000021728	A	AU 2000-21728	19991210
EP 1137664	A2	EP 1999-966101	19991210
		WO 1999-US29289	19991210
JP 2002531113	W	WO 1999-US29289	19991210
		JP 2000-586751	19991210

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000021728	A	Based on	WO 2000034308
EP 1137664	A2	Based on	WO 2000034308
JP 2002531113	W	Based on	WO 2000034308

PRIORITY APPLN. INFO: US 1998-111701P 19981210
AN 2000-431269 [37] WPIDS

AB WO 200034308 A UPAB: 20000807
NOVELTY - Protein transduction system (I) comprising a fusion protein (F) has a covalently linked protein transduction domain (D1) and cytotoxic domain (D2).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a substantially pure (F);

(2) a nucleic acid segment (II) encoding (F);

(3) a DNA vector comprising (II);

(4) screening for a candidate compound to inhibit a pathogens-specific protease comprising transducing (F) into a cell population, expressing the protease by infecting with the pathogen, contacting the protease with (F) to produce a cytotoxin and modulating the protease;

(5) a kit comprising (I);

(6) introducing (F) into a cell by isolating (F) from a host cell, misfolding (F) and transducing it into the cell; and

(7) a protein transduction domain represented by or comprising at least a peptide of the following formulae:

B1-X1-X2-X3-B2-X4-X5-B3 or B1-X1-X2-B2-B3-X3-X4-B4,

where,

B1 - B3 = basic amino acid; and

X1 - X5 = alpha -helix enhancing amino acids.

ACTIVITY - Virucide; Anti-HIV; Hepatotropic; Antiinflammatory; Protozoacide.

Jurkat T-cells transduced with purified p16 fusion proteins were infected by HIV and control cells transduced with vector not containing a HIV protease cleavage site. Result show efficient cleavage of p16 fusion proteins encoded by vectors containing HIV cleavage sites compared to control.

MECHANISM OF ACTION - Fusion protein (cytotoxin)-transduction enhancer.

USE - (I) is useful for treating pathogen infection in mammals, infections such as CMV, HSV-1, HCV, KSHV, yellow fever virus, flavivirus or rhinovirus, retroviral infections such as HIV-1, HIV-2, HTVL-3 and/or LAV, plasmodial infections associated with P.faciparum, P.vivax, P.ovale, P.malariae, cancer especially prostate cancer in which diseased cells express of property which can be targeted, such as elevated level of heavy metals e.g. zinc which promotes an inactive monomeric protein to become an active dimer. (I) is also useful for suppressing tumors by administering (I) comprising a cell cycle inhibitor such as p16, p27 or Cdk2DN along with a chemotherapeutic agent such as a DNA synthesis inhibitor that interacts in the S-phase of a targeted cell or a DNA damage initiator and thus promoting apoptosis (claimed).

ADVANTAGE - (D1) increases transduction efficiency of a protein by 5-10 fold and up to 100 fold as determined from intracellular concentrations of (D1) (claimed).

Dwg.0/21

L6 ANSWER 34 OF 35 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: 1999-590695 [50] WPIDS

DOC. NO. NON-CPI: N1999-435671

DOC. NO. CPI: C1999-172440

TITLE: Production of cytotoxic heteromeric protein combinatorial libraries, useful for ability to specifically bind to and kill a target cell.

DERWENT CLASS: B04 D16 S03

INVENTOR(S): BRAY, M R; GARIEPY, J

PATENT ASSIGNEE(S): (UYHE-N) UNIV HEALTH NETWORK; (ONTA-N) ONTARIO CANCER INST

COUNTRY COUNT: 83

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9940185	A1	19990812	(199950)*	EN	61
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
CA 2222993	A1	19990804	(200004)	EN	
AU 9915530	A	19990823	(200005)		
EP 1051482	A1	20001115	(200059)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
JP 2002503453	W	20020205	(200212)		60
AU 769824	B	20040205	(200413)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9940185	A1	WO 1998-CA1137	19981208
CA 2222993	A1	CA 1998-2222993	19980204
AU 9915530	A	AU 1999-15530	19981208
EP 1051482	A1	EP 1998-959689	19981208
		WO 1998-CA1137	19981208
JP 2002503453	W	WO 1998-CA1137	19981208
		JP 2000-530599	19981208
AU 769824	B	AU 1999-15530	19981208

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9915530	A	Based on	WO 9940185
EP 1051482	A1	Based on	WO 9940185
JP 2002503453	W	Based on	WO 9940185
AU 769824	B	Previous Publ.	AU 9915530
		Based on	WO 9940185

PRIORITY APPLN. INFO: CA 1998-2222993 19980204

AN 1999-590695 [50] WPIDS

AB WO 9940185 A UPAB: 19991201

NOVELTY - A **binding** subunit of a wild type heteromeric cytotoxic protein is **mutated** to create a library of microorganism clones producing mutant proteins where are then screened for their ability to specifically bind to and kill a target cell.

DETAILED DESCRIPTION - A method for identifying cytotoxic mutant proteins capable of binding to a target cell comprises:

(a) selecting a heteromeric protein toxin having a toxic subunit and a binding subunit;

(**b**) generating a library of microorganism clones producing variant protein toxins of the heteromeric protein toxin by incorporating **mutations** into the **binding** subunit DNA of the heteromeric protein toxin; and

(c) screening the variant protein toxins of the library against the target cell by isolating clones or pools of clones producing the variant protein toxins, treating preparations of the target cells with the variant protein toxins and selecting a cytotoxic mutant protein or pool of cytotoxic mutant proteins that inhibit or kill the target cell.

INDEPENDENT CLAIMS are also included for the following:

(1) a method of killing or inhibiting a target cell comprising

treating the target cell with the cytotoxic mutant protein or pool of proteins from above;

(2) a method for identifying therapeutic proteins having binding specificity for a target cell; and

(3) a method for constructing diagnostic probes for detecting the presence of a cell surface marker.

USE - Cytotoxic mutant proteins identified by the method can be used to identify therapeutic proteins and medicaments having binding specificity for a target cell. The cytotoxic mutants can also be used to construct diagnostic probes for detecting the presence of cell surface markers. These medicaments can be used to target medicines to target cells in host organisms. (All Claimed).

Dwg.0/6

L6 ANSWER 35 OF 35 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: 1999-132157 [11] WPIDS

DOC. NO. CPI: C1999-038688

TITLE: New chimeric constructs of **Shiga toxin** B fragment with polypeptide or nucleic acid - to provide retrograde transport in cells, particularly for presentation of antigenic epitopes or for restoration of defective intracellular transport.

DERWENT CLASS: B04 D16

INVENTOR(S): GOUD, B; JOHANNES, L

PATENT ASSIGNEE(S): (CNRS) CENT NAT RECH SCI; (CURI-N) INST CURIE; (CNRS) CNRS CENT NAT RECH SCI; (GOUD-I) GOUD B; (JOHA-I) JOHANNES L

COUNTRY COUNT: 83

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9903881	A2	19990128	(199911)*	FR	34
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
FR 2766193	A1	19990122	(199911)		
AU 9888124	A	19990210	(199925)		
EP 1017715	A2	20000712	(200036)	FR	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
CN 1272882	A	20001108	(200114)		
JP 2001510030	W	20010731	(200148)		35
AU 750367	B	20020718	(200258)		
US 6613882	B1	20030902	(200359)		
US 2004047883	A1	20040311	(200419)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9903881	A2	WO 1998-FR1573	19980717
FR 2766193	A1	FR 1997-9185	19970718
AU 9888124	A	AU 1998-88124	19980717
EP 1017715	A2	EP 1998-939705	19980717
		WO 1998-FR1573	19980717
CN 1272882	A	CN 1998-808796	19980717
JP 2001510030	W	WO 1998-FR1573	19980717
		JP 2000-503103	19980717
AU 750367	B	AU 1998-88124	19980717

US 6613882	B1 Cont of	WO 1998-FR1573	19980717
		US 2000-484471	20000118
US 2004047883	A1 Cont of	WO 1998-FR1573	19980717
	Cont of	US 2000-484471	20000118
		US 2003-443614	20030521

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9888124	A	Based on	WO 9903881
EP 1017715	A2	Based on	WO 9903881
JP 2001510030	W	Based on	WO 9903881
AU 750367	B	Previous Publ.	AU 9888124
		Based on	WO 9903881
US 2004047883	A1	Cont of	US 6613882

PRIORITY APPLN. INFO: FR 1997-9185 19970718
AN 1999-132157 [11] WPIDS
AB WO 9903881 A UPAB: 19990316
New chimeric sequence of formula B-X (I), where B = fragment B of **Shiga toxin** or its functional equivalent X = one or more polypeptides, provided that the total length of (I) is compatible with retrograde transport.

Also new are: (1) chimeras (Ia) of B fragment with one or more polynucleotides X', containing a sequence that encodes X that is to be expressed.

USE - (I) and (Ia) are used: (a) for antigenic presentation of epitopes to cells of the immune system, particularly (i) to stimulate immune defences against viral, parasitic or bacterial infections or cancer-associated antigens: or (**b**) to suppress or eliminate an autoimmune response, and (ii) to restore intracellular transport of proteins that have a **mutation** in the chaperone **binding** site, particularly for treatment of cystic fibrosis (claimed).

ADVANTAGE - Attachment of X or X' to B (which serves as vector) directs its transport to the endoplasmic reticulum (RE) and allows for subsequent maturation and optionally presentation at the membrane surface. Use of (I) and (Ia) eliminates the need for (retro)viral vectors.
Dwg.0/5

=> FIL STNGUIDE
=>

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	151.74	151.95

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-2.94	-2.94

FILE 'STNGUIDE' ENTERED AT 17:00:45 ON 05 AUG 2004

FILE CONTAINS CURRENT INFORMATION.
LAST RELOADED: Jul 30, 2004 (20040730/UP).

=>
=> ?toxin and bind? and mutat? and resistan?
?TOXIN IS NOT A RECOGNIZED COMMAND

```
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s ?toxin and bind? and mutat? and resistan?
LEFT TRUNCATION IGNORED FOR '?TOXIN' FOR FILE 'STNGUIDE'
             0 TOXIN
             1 BIND?
             1 MUTAT?
             8 RESISTAN?
L7           0 ?TOXIN AND BIND? AND MUTAT? AND RESISTAN?
Left truncation is not valid in the specified search field in the
specified file.  The term has been searched without left truncation.
Examples: '?TERPEN?' would be searched as 'TERPEN?' and '?FLAVONOID'
would be searched as 'FLAVONOID.'

If you are searching in a field that uses implied proximity, and you
used a truncation symbol after a punctuation mark, the system may
interpret the truncation symbol as being at the beginning of a term.
Implied proximity is used in search fields indexed as single words,
for example, the Basic Index.

=> file medline biosis embase caplus wpids
COST IN U.S. DOLLARS                              SINCE FILE      TOTAL
                                                     ENTRY    SESSION
FULL ESTIMATED COST                                   1.74     153.69

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)        SINCE FILE      TOTAL
                                                     ENTRY    SESSION
CA SUBSCRIBER PRICE                                   0.00      -2.94

FILE 'MEDLINE' ENTERED AT 17:18:15 ON 05 AUG 2004

FILE 'BIOSIS' ENTERED AT 17:18:15 ON 05 AUG 2004
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FILE 'EMBASE' ENTERED AT 17:18:15 ON 05 AUG 2004
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FILE 'CAPLUS' ENTERED AT 17:18:15 ON 05 AUG 2004
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
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FILE 'WPIDS' ENTERED AT 17:18:15 ON 05 AUG 2004
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=> s ?toxin and bind? and mutat? and resistan?
L8          761 ?TOXIN AND BIND? AND MUTAT? AND RESISTAN?

=>

=> s l8 and py>1999
L9          293 L8 AND PY>1999

=> l8 not l9
L8 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s l8 not l9
```

L10 468 L8 NOT L9

=> d scan

L10 468 ANSWERS BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI **Mutational** analysis of VAMP domains implicated in Ca-2+-induced insulin exocytosis.
IT Miscellaneous Descriptors
BIOCHEMISTRY AND BIOPHYSICS; CALCIUM ION-INDUCED INSULIN EXOCYTOSIS; CELLUBREVIN; ENDOCRINE SYSTEM; INSULIN; INSULIN-CONTAINING SECRETORY GRANULES; ISLETS OF LANGERHANS; **MUTATIONAL** ANALYSIS; NEUROTOXINS; PANCREAS; SYNAPTIC VESICLE TARGETING; VESICLE-ASSOCIATED MEMBRANE PROTEIN-2

HOW MANY MORE ANSWERS DO YOU WISH TO SCAN? (1):4

L10 468 ANSWERS BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI CELL AND SPECIES DIFFERENCES IN METABOLIC ACTIVATION OF CHEMICAL CARCINOGENS.
IT Miscellaneous Descriptors
LIVER CANCER **AFLATOXIN** B-1 N NITROSODIMETHYLAMINE BENZO-A-PYRENE HUMAN HEPATOCYTES RAT HEPATOCYTES MOUSE HEPATOCYTES HUMAN PULMONARY ALVEOLAR MACROPHAGES CHINESE HAMSTER V-79 CELLS

L10 468 ANSWERS CAPLUS COPYRIGHT 2004 ACS on STN
CC 10-2 (Microbial, Algal, and Fungal Biochemistry)
TI Secretion of FK506/FK520 and rapamycin by Streptomyces inhibits the growth of competing Saccharomyces cerevisiae and Cryptococcus neoformans
ST Streptomyces secretion protein FK506 FK520 rapamycin
IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(FK506; secretion of proteins FK506/FK520 and rapamycin by Streptomyces inhibits growth of competing Saccharomyces cerevisiae and Cryptococcus neoformans)
IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(FK520; secretion of proteins FK506/FK520 and rapamycin by Streptomyces inhibits growth of competing Saccharomyces cerevisiae and Cryptococcus neoformans)
IT Cryptococcus neoformans
Growth, microbial
Microbial ecology
Saccharomyces cerevisiae
Secretion (process)
(secretion of proteins FK506/FK520 and rapamycin by Streptomyces inhibits growth of competing Saccharomyces cerevisiae and Cryptococcus neoformans)
IT 53123-88-9, Rapamycin
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(secretion of proteins FK506/FK520 and rapamycin by Streptomyces inhibits growth of competing Saccharomyces cerevisiae and Cryptococcus neoformans)

L10 468 ANSWERS CAPLUS COPYRIGHT 2004 ACS on STN
CC 6-3 (General Biochemistry)
Section cross-reference(s): 3, 10

TI HKR1 encodes a cell surface protein that regulates both cell wall β-glucan synthesis and budding pattern in the yeast Saccharomyces cerevisiae

ST Saccharomyces gene HKR1 cell surface protein

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(HKR1; gene HKR1 encodes a cell surface protein that regulates both cell wall β-glucan synthesis and budding pattern in the yeast Saccharomyces cerevisiae)

IT Cytoplasm
(Saccharomyces cerevisiae gene HKR1 encodes a type 1 membrane protein that contains a calcium-**binding** consensus sequence (EF hand motif) in the cytoplasmic domain)

IT Cell wall
(disruption of the 3' part of the coding region of HKR1 significantly reduced β-1,3-glucan synthase activity and the amount of β-1,3-glucan in the cell wall and altered the axial budding pattern of haploid Saccharomyces cerevisiae cells)

IT Saccharomyces cerevisiae
(gene HKR1 encodes a cell surface protein that regulates both cell wall β-glucan synthesis and budding pattern in the yeast Saccharomyces cerevisiae)

IT Escherichia coli
(immunofluorescence microscopy with an antibody raised against Saccharomyces cerevisiae Hkr1p expressed in Escherichia coli revealed that Hkr1p was predominantly localized on the cell surface)

IT Microorganism development
(budding, disruption of the 3' part of the coding region of HKR1 significantly reduced β-1,3-glucan synthase activity and the amount of β-1,3-glucan in the cell wall and altered the axial budding pattern of haploid Saccharomyces cerevisiae cells)

IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(cell surface-associated, gene HKR1 encodes a cell surface protein that regulates both cell wall β-glucan synthesis and budding pattern in the yeast Saccharomyces cerevisiae)

IT **Mutation**
(deletion, although the null **mutation** of HKR1 is lethal, disruption of the 3' part of the coding region, which would result in deletion of the cytoplasmic domain of Hkr1p, did not affect the viability of yeast cells)

IT Peptides, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(signal, the cell surface localization of Saccharomyces cerevisiae Hkr1p required the N-terminal signal sequence because the C-terminal half of Hkr1p was detected uniformly in the cells)

IT 7440-70-2, Calcium, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Saccharomyces cerevisiae gene HKR1 encodes a type 1 membrane protein that contains a calcium-**binding** consensus sequence (EF hand motif) in the cytoplasmic domain)

IT 9037-30-3, β-1,3-Glucan synthase
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(disruption of the 3' part of the coding region of HKR1 significantly reduced β-1,3-glucan synthase activity and the amount of β-1,3-glucan in the cell wall and altered the axial budding pattern of haploid Saccharomyces cerevisiae cells)

IT 9051-97-2

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(gene HKR1 encodes a cell surface protein that regulates both cell wall β-glucan synthesis and budding pattern in the yeast Saccharomyces cerevisiae)

L10 468 ANSWERS CAPLUS COPYRIGHT 2004 ACS on STN
CC 5-4 (Agrochemical Bioregulators)
TI Drosophila sodium channel **mutations** affect pyrethroid sensitivity
ST Drosophila sodium channel **mutation** pyrethroid; review Drosophila sodium channel **mutation** pyrethroid
IT **Mutation**
(in sodium channels of Drosophila, pyrethroid sensitivity in relation to)
IT Drosophila melanogaster
(sodium channel **mutations** in, pyrethroid sensitivity in relation to)
IT Pyrethrins and Pyrethroids
RL: BIOL (Biological study)
(Drosophila sensitivity to, sodium channel **mutations** effect on)
IT Ion channel
(sodium, **mutations** in, in Drosophila, pyrethroid sensitivity in relation to)
IT 7440-23-5, Sodium, biological studies
RL: BIOL (Biological study)
(channels for, **mutations** in Drosophila, pyrethroid sensitivity in relation to)

HOW MANY MORE ANSWERS DO YOU WISH TO SCAN? (1):end

=> 1
1 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> t ti l10 1-5

L10 ANSWER 1 OF 468 MEDLINE on STN
TI Control of DNA replication and cell proliferation in eukaryotes by aphidicolin.

L10 ANSWER 2 OF 468 MEDLINE on STN
TI Overexpression of stimulatory G protein alpha-subunit is a hallmark of most human somatotrophic pituitary tumours and is associated with **resistance** to GH-releasing hormone.

L10 ANSWER 3 OF 468 MEDLINE on STN
TI An Asp79Asn **mutation** of the alpha2A-adrenoceptor interferes equally with agonist activation of individual Gialpha-family G protein subtypes.

L10 ANSWER 4 OF 468 MEDLINE on STN
TI Lethal paralysis of Caenorhabditis elegans by Pseudomonas aeruginosa.

L10 ANSWER 5 OF 468 MEDLINE on STN
TI Point-**mutations** related to the loss of **batrachotoxin binding** abolish the **grayanotoxin** effect in Na(+) channel isoforms.

```
=> d his

     (FILE 'HOME' ENTERED AT 16:29:01 ON 05 AUG 2004)

     FILE 'MEDLINE, BIOSIS, EMBASE, CAPLUS, WPIDS' ENTERED AT 16:29:27 ON 05
     AUG 2004
L1          7735 S ((SHIGA (W) LIKE) OR SHIGA) (S) TOXIN
L2            67 S L1 AND MUTAT? (S) (B OR BINDING)
L3            37 DUP REM L2 (30 DUPLICATES REMOVED)
L4             2 S L3 AND RESISTAN?
L5             0 S L4 NOT L3
L6            35 S L3 NOT L4

     FILE 'STNGUIDE' ENTERED AT 17:00:45 ON 05 AUG 2004
L7             0 S ?TOXIN AND BIND? AND MUTAT? AND RESISTAN?

     FILE 'MEDLINE, BIOSIS, EMBASE, CAPLUS, WPIDS' ENTERED AT 17:18:15 ON 05
     AUG 2004
L8           761 S ?TOXIN AND BIND? AND MUTAT? AND RESISTAN?
L9           293 S L8 AND PY>1999
L10          468 S L8 NOT L9

=> s (mutat? (s) bind?) and l10
L11          180 (MUTAT? (S) BIND?) AND L10

=> t ti l11 1-10

L11  ANSWER 1 OF 180      MEDLINE on STN
TI   Point-mutations related to the loss of batrachotoxin
     binding abolish the grayanotoxin effect in Na(+) channel
     isoforms.

L11  ANSWER 2 OF 180      MEDLINE on STN
TI   Point mutations at N434 in D1-S6 of mu1 Na(+) channels modulate
     binding affinity and stereoselectivity of local anesthetic
     enantiomers.

L11  ANSWER 3 OF 180      MEDLINE on STN
TI   betagamma dimers derived from Go and Gi proteins contribute different
     components of adrenergic inhibition of Ca2+ channels in rat sympathetic
     neurones.

L11  ANSWER 4 OF 180      MEDLINE on STN
TI   Batrachotoxin-resistant Na+ channels derived from
     point mutations in transmembrane segment D4-S6.

L11  ANSWER 5 OF 180      MEDLINE on STN
TI   Integrative model for binding of Bacillus thuringiensis toxins
     in susceptible and resistant larvae of the diamondback moth
     (Plutella xylostella).

L11  ANSWER 6 OF 180      MEDLINE on STN
TI   Mutation of a conserved serine residue in a quinolone-
     resistant type II topoisomerase alters the enzyme-DNA and drug
     interactions.

L11  ANSWER 7 OF 180      MEDLINE on STN
TI   Functional characterization of mongoose nicotinic acetylcholine receptor
     alpha-subunit: resistance to alpha-bungarotoxin and
     high sensitivity to acetylcholine.
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L11 ANSWER 8 OF 180 MEDLINE on STN
TI Local anesthetic block of **batrachotoxin-resistant** muscle Na+ channels.

L11 ANSWER 9 OF 180 MEDLINE on STN
TI Extrapore residues of the S5-S6 loop of domain 2 of the voltage-gated skeletal muscle sodium channel (rSkM1) contribute to the mu-**conotoxin** GIIIA **binding** site.

L11 ANSWER 10 OF 180 MEDLINE on STN
TI Point **mutations** in segment I-S6 render voltage-gated Na+ channels **resistant** to **batrachotoxin**.

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L11 ANSWER 11 OF 180 MEDLINE on STN
TI Global variation in the genetic and biochemical basis of diamondback moth **resistance** to Bacillus thuringiensis.

L11 ANSWER 12 OF 180 MEDLINE on STN
TI The biochemical effect of the naturally occurring Trp64-->Arg **mutation** on human beta3-adrenoceptor activity.

L11 ANSWER 13 OF 180 MEDLINE on STN
TI A superantigen-antibody fusion protein for T-cell immunotherapy of human B-lineage malignancies.

L11 ANSWER 14 OF 180 MEDLINE on STN
TI A mu-**conotoxin**-insensitive Na+ channel mutant: possible localization of a **binding** site at the outer vestibule.

L11 ANSWER 15 OF 180 MEDLINE on STN
TI Killer-**toxin-resistant** kre12 mutants of Saccharomyces cerevisiae: genetic and biochemical evidence for a secondary K1 membrane receptor.

L11 ANSWER 16 OF 180 MEDLINE on STN
TI Two subsites in the **binding** domain of the acetylcholine receptor: an aromatic subsite and a proline subsite.

L11 ANSWER 17 OF 180 MEDLINE on STN
TI Isolation and characterization of a Clostridium botulinum C2 **toxin -resistant** cell line: evidence for possible involvement of the cellular C2II receptor in growth regulation.

L11 ANSWER 18 OF 180 MEDLINE on STN
TI A unique amino acid of the Drosophila GABA receptor with influence on drug sensitivity by two mechanisms.

L11 ANSWER 19 OF 180 MEDLINE on STN
TI Identification of an amino acid substitution in human alpha 1 Na,K-ATPase which confers differentially reduced affinity for two related cardiac glycosides.

L11 ANSWER 20 OF 180 MEDLINE on STN
TI Post-repolarization block of cloned sodium channels by **saxitoxin** : the contribution of pore-region amino acids.

L11 ANSWER 21 OF 180 MEDLINE on STN
TI Mapping **mutations** in genes encoding the two large subunits of Drosophila RNA polymerase II defines domains essential for basic

transcription functions and for proper expression of developmental genes.

L11 ANSWER 22 OF 180 MEDLINE on STN
TI Characterization of adenylate cyclase **toxin** from a mutant of Bordetella pertussis defective in the activator gene, cyaC.

L11 ANSWER 23 OF 180 MEDLINE on STN
TI Inhibition of HIV-1 RNA production by the diphtheria **toxin** -related IL-2 fusion proteins DAB486IL-2 and DAB389IL-2.

L11 ANSWER 24 OF 180 MEDLINE on STN
TI Translocation mediated by domain II of Pseudomonas **exotoxin** A: transport of barnase into the cytosol.

L11 ANSWER 25 OF 180 MEDLINE on STN
TI K1 killer **toxin**, a pore-forming protein from yeast.

L11 ANSWER 26 OF 180 MEDLINE on STN
TI Identification of diphtheria **toxin** receptor and a nonproteinous diphtheria **toxin-binding** molecule in Vero cell membrane.

L11 ANSWER 27 OF 180 MEDLINE on STN
TI X-rays **mutate** human lymphoblast cells at genetic loci that should respond only to point mutagens.

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TI Point-**mutations** related to the loss of **batrachotoxin binding** abolish the **grayanotoxin** effect in Na+ channel isoforms.

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TI Point **mutations** at N434 in D1-S6 of mu1 Na+ channels modulate **binding** affinity and stereoselectivity of local anesthetic enantiomers.

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TI betagamma dimers derived from Go and Gi proteins contribute different components of adrenergic inhibition of Ca2+ channels in rat sympathetic neurones.

L11 ANSWER 31 OF 180 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Aggregation of Bacillus thuringiensis Cry1A toxins upon **binding** to target insect larval midgut vesicles.

L11 ANSWER 32 OF 180 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI **Batrachotoxin-resistant** Na+ channels derived from point **mutations** in transmembrane segment D4-S6.

L11 ANSWER 33 OF 180 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Integrative model for **binding** of Bacillus thuringiensis toxins in susceptible and **resistant** larvae of the diamondback moth (Plutella xylostella).

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TI **Mutation** of a conserved serine residue in a quinolone-**resistant** type II topoisomerase alters the enzyme-DNA and drug interactions.

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TI Local anesthetic block of **batrachotoxin-resistant** muscle Na+ channels.

L11 ANSWER 36 OF 180 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Functional characterization of mongoose nicotinic acetylcholine receptor alpha-subunit: **Resistance** to alpha-**bungarotoxin** and high sensitivity to aceylcholine.

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TI Extrapore residues of the S5-S6 loop of domain 2 of the voltage-gated skeletal muscle sodium channel (rSkM1) contribute to the mu-**conotoxin** GIIIA **binding** site.

L11 ANSWER 38 OF 180 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Point **mutations** in segment I-S6 render voltage-gated Na+ channels **resistant** to **batrachotoxin**.

L11 ANSWER 39 OF 180 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Global variation in the genetic and biochemical basis of diamondback moth **resistance** to Bacillus thuringiensis.

L11 ANSWER 40 OF 180 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI The biochemical effect of the naturally occurring Trp64 fwdarw Arg **mutation** on human beta-3-adrenoceptor activity.

L11 ANSWER 41 OF 180 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI A superantigen-antibody fusion protein for T-cell immunotherapy of human B-lineage malignancies.

L11 ANSWER 42 OF 180 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Killer-**toxin-resistant** krel2 mutants of Saccharomyces cerevisiae: Genetic and biochemical evidence for a secondary K1 membrane receptor.

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TI Two subsites in the **binding** domain of the acetylcholine receptor: An aromatic subsite and a proline subsite.

L11 ANSWER 44 OF 180 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Isolation and Characterization of a Clostridium botulinum C2 **Toxin -Resistant** Cell Line: Evidence for Possible Involvement of the Cellular C2II Receptor in Growth Regulation.

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TI A unique amino acid of the Drosophila GABA receptor with influence on drug sensitivity by two mechanisms.

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TI Characterization of adenylate cyclase **toxin** from a mutant of Bordetella pertussis defective in the activator gene, cyaC.

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TI Inhibition of HIV-1 RNA production by the diphtheria **toxin** -related IL-2 fusion proteins DAB-486IL-2 and DAB-389IL-2.

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TI SPECIFIC **BINDING** OF ADH AND CAMP IN THE KIDNEY MEDULLA OF THE ADH-**RESISTANT** MICE.

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TI βγ dimers derived from G0 and G1 proteins contribute different components of adrenergic inhibition of Ca2+ channels in rat sympathetic neurones.

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TI **Batrachotoxin-resistant** Na+ channels derived from point **mutations** in transmembrane segment D4-S6.

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TI Aggregation of Bacillus thuringiensis Cry1A toxins upon **binding** to target insect larval midgut vesicles.

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TI New actin mutants allow further characterization of the nucleotide **binding** cleft and drug **binding** sites.

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TI Point **mutations** in segment I-S6 render voltage-gated Na+ channels **resistant** to **batrachotoxin.**

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TI Gene probes used for genetic profiling in healthcare screening and planning

L11 ANSWER 140 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI An Asp79Asn **mutation** of the α2A-adrenoceptor interferes equally with agonist activation of individual Giα-family G protein subtypes

L11 ANSWER 141 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Activation of constitutive 5-hydroxytryptamine1B receptor by a series of **mutations** in the BBXXB motif: positioning of the third intracellular loop distal junction and its Goα protein interactions

L11 ANSWER 142 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Point **mutations** at N434 in D1-S6 of μ1 Na+ channels modulate **binding** affinity and stereoselectivity of local anesthetic enantiomers

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TI βγ Dimers derived from Go and Gi proteins contribute different components of adrenergic inhibition of Ca2+ channels in rat sympathetic

neurons

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TI Modulation of 5-HT1A receptor signalling by point-**mutation** of cysteine351 in the rat Gαo protein

L11 ANSWER 145 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Aggregation of Bacillus thuringiensis Cry1A toxins upon **binding** to target insect larval midgut vesicles

L11 ANSWER 146 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI **Batrachotoxin-resistant** Na+ channels derived from point **mutations** in transmembrane segment D4-S6

L11 ANSWER 147 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Integrative model for **binding** of Bacillus thuringiensis toxins in susceptible and **resistant** larvae of the diamondback moth (Plutella xylostella)

L11 ANSWER 148 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI **Mutation** of a conserved serine residue in a quinolone-**resistant** type II topoisomerase alters the enzyme-DNA and drug interactions

L11 ANSWER 149 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Local anesthetic block of **batrachotoxin-resistant** muscle Na+ channels

L11 ANSWER 150 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Functional characterization of mongoose nicotinic acetylcholine receptor α-subunit: **resistance** to α- **bungarotoxin** and high sensitivity to acetylcholine

=> t ti l11 151-180

L11 ANSWER 151 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Extrapore residues of the S5-S6 loop of domain 2 of the voltage-gated skeletal muscle sodium channel (rSKM1) contribute to the μ-**conotoxin** GIIIA **binding** site

L11 ANSWER 152 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Quantitative analysis of a cysteine351glycine **mutation** in the G protein Gi1α: effect on α2A-adrenoceptor-Gi1α fusion protein activation

L11 ANSWER 153 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Point **mutations** in segment I-S6 render voltage-gated Na+ channels **resistant** to **batrachotoxin**

L11 ANSWER 154 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Global variation in the genetic and biochemical basis of diamondback moth **resistance** to Bacillus thuringiensis

L11 ANSWER 155 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI The biochemical effect of the naturally occurring Trp64→Arg **mutation** on human β3-adrenoceptor activity

L11 ANSWER 156 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI A Cysteine-3 to Serine **Mutation** of the G-Protein Gi1α Abrogates Functional Activation by the α2A-Adrenoceptor but Not Interactions with the βγ Complex

L11 ANSWER 157 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Identification of **mutations** at DNA topoisomerase I responsible for camptothecin **resistance**

L11 ANSWER 158 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI A superantigen-antibody fusion protein for T-cell immunotherapy of human B-lineage malignancies

L11 ANSWER 159 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Killer-**toxin**-**resistant** kre12 mutants of Saccharomyces cerevisiae. Genetic and biochemical evidence for a secondary K1 membrane receptor

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TI Two subsites in the **binding** domain of the acetylcholine receptor: An aromatic subsite and a proline subsite

L11 ANSWER 161 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Why puffer fishes are not intoxicated by their carring **tetrodotoxin**?

L11 ANSWER 162 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Molecular biological investigations of the property of sodium channels of Blattella germanica.

L11 ANSWER 163 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Isolation and characterization of a Clostridium botulinum C2 **toxin** -**resistant** cell line: evidence for possible involvement of the cellular C2II receptor in growth regulation

L11 ANSWER 164 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI A Mutant of Protein Phosphatase-1 that Exhibits Altered **Toxin** Sensitivity

L11 ANSWER 165 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI A unique amino acid of the Drosophila GABA receptor with influence on drug sensitivity by two mechanisms

L11 ANSWER 166 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Identification of an amino acid substitution in human α1 Na,K-ATPase which confers differentially reduced affinity for two related cardiac glycosides

L11 ANSWER 167 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Mapping **mutations** in genes encoding the two large subunits of Drosophila RNA polymerase II defines domains essential for basic transcription functions and for proper expression of developmental genes

L11 ANSWER 168 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Characterization of adenylate cyclase **toxin** from a mutant of Bordetella pertussis defective in the activator gene, cyaC

L11 ANSWER 169 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Inhibition of HIV-1 RNA production by the diphtheria **toxin** -related IL-2 fusion proteins DAB486IL-2 and DAB389IL-2

L11 ANSWER 170 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Defective guanyl nucleotide-**binding** protein βγ subunits in a forskolin-**resistant** mutant of the Y1 adrenocortical cell line

L11 ANSWER 171 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Insertional mutagenesis of Bordetella pertussis adenylate cyclase

L11 ANSWER 172 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Activating and inactivating **mutations** of the α subunit of Gi2 protein have opposite effects on proliferation of NIH 3T3 cells

L11 ANSWER 173 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Drosophila sodium channel **mutations** affect pyrethroid sensitivity

L11 ANSWER 174 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI A Drosophila **mutation** that reduces sodium channel number confers **resistance** to pyrethroid insecticides

L11 ANSWER 175 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Identification of diphtheria **toxin** receptor and a nonproteinous diphtheria **toxin-binding** molecule in Vero cell membrane

L11 ANSWER 176 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI X-rays **mutate** human lymphoblast cells at genetic loci that should respond only to point mutagens

L11 ANSWER 177 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Mutagenesis in Streptococcus pneumoniae (Pneumococcus) by transformation with DNA modified by the carcinogen-mutagen, **aflatoxin** B1

L11 ANSWER 178 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI **Aflatoxin** B1 mutagenesis, DNA **binding**, and adduct formation in Salmonella typhimurium

L11 ANSWER 179 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
TI Amanitin **binding** to RNA polymerase II in α-amanitin-**resistant** rat myoblast mutants

L11 ANSWER 180 OF 180 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI New nucleotide primers - useful for detecting methicillin-**resistant** or toxic shock syndrome-**toxin** producing Staphylococci.

=> d ibib abs l11 67,74,145,160

L11 ANSWER 67 OF 180 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 1999132769 EMBASE
TITLE: Integrative model for **binding** of Bacillus thuringiensis toxins in susceptible and **resistant** larvae of the diamondback moth (Plutella xylostella).
AUTHOR: Ballester V.; Granero F.; Tabashnik B.E.; Malvar T.; Ferre J.
CORPORATE SOURCE: J. Ferre, Departament de Genetica, Universitat de Valencia, 46100 Burjassot, Valencia, Spain. juan.ferre@uv.es
SOURCE: Applied and Environmental Microbiology, (1999) 65/4 (1413-1419).
Refs: 49
ISSN: 0099-2240 CODEN: AEMIDF
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English

SUMMARY LANGUAGE: English

AB Insecticidal crystal proteins from Bacillus thuringiensis in sprays and transgenic crops are extremely useful for environmentally sound pest management, but their long-term efficacy is threatened by evolution of **resistance** by target pests. The diamondback moth (Plutella xylostella) is the first insect to evolve **resistance** to B. thuringiensis in open-field populations. The only known mechanism of **resistance** to B. thuringiensis in the diamondback moth is reduced **binding** of **toxin** to midgut **binding** sites. In the present work we analyzed competitive **binding** of B. thuringiensis toxins Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F to brush border membrane vesicles from larval midguts in a susceptible strain and in **resistant** strains from the Philippines, Hawaii, and Pennsylvania. Based on the results, we propose a model for **binding** of B. thuringiensis crystal proteins in susceptible larvae with two **binding** sites for Cry1Aa, one of which is shared with Cry1Ab, Cry1AC, and Cry1F. Our results show that the common **binding** site is altered in each of the three **resistant** strains. In the strain from the Philippines, the alteration reduced **binding** of Cry1Ab but did not affect **binding** of the other crystal proteins. In the **resistant** strains from Hawaii and Pennsylvania, the alteration affected **binding** of Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F. Previously reported evidence that a single **mutation** can confer **resistance** to Cry1Ab, Cry1Ac, and Cry1F corresponds to expectations based on the **binding** model. However, the following two other observations do not: the **mutation** in the Philippines strain affected **binding** of only Cry1Ab, and one **mutation** was sufficient for **resistance** to Cry1Aa. The imperfect correspondence between the model and observations suggests that reduced **binding** is not the only mechanism of **resistance** in the diamondback moth and that some, but not all, patterns of **resistance** and cross-**resistance** can be predicted correctly from the results of competitive **binding** analyses of susceptible strains.

L11 ANSWER 74 OF 180 EMBASE on STN

ACCESSION NUMBER: 1998300700 EMBASE

TITLE: **Toxin binding** site of the diphtheria **toxin** receptor: Loss and gain of diphtheria **toxin binding** of monkey and mouse heparin-**binding**, epidermal growth factor-like growth factor precursors by reciprocal site-directed mutagenesis.

AUTHOR: Cha J.-H.; Brooke J.S.; Eidels L.

CORPORATE SOURCE: L. Eidels, Department of Microbiology, Univ. Texas Southwestern Med. Center, 600 Harry Hines Boulevard, Dallas, TX 75235-9048, United States. LEIDEL@MEDNET.SWMED.EDU

SOURCE: Molecular Microbiology, (1998) 29/5 (1275-1284). Refs: 30 ISSN: 0950-382X CODEN: MOMIEE

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The transmembrane precursor of the monkey (Mk) heparin-**binding**, epidermal growth factor-like growth factor (proHB-EGF) functions as a diphtheria **toxin** (DT) receptor, whereas the mouse (Ms) precursor does not. Previously, using chimeric Ms/Mk precursors, we have shown that DT **resistance** of cells bearing Ms proHB-EGF may be accounted for

by several amino acid substitutions between residues 122 and 148 within the EGF-like domain and that Glu-141 is an important amino acid residue for DT **binding**. In this study, reciprocal site-directed mutagenesis was performed on the major non-conserved residues in the region of 122-148, alone or in combination, between Mk and Ms precursors to identify more precisely which amino acid residues are important for DT **binding**. Two approaches were used. The first, more traditional approach was to destroy DT sensitivity and **binding** of Mk proHB-EGF by substitution(s) with the corresponding Ms residue(s). From the single **mutations**, the greatest loss of DT sensitivity was observed with Mk/Glu-141His (approximately 4000-fold) and the next greatest with Mk/Ile-133Lys (approximately fourfold). The double **mutations** Mk/Leu-177Phe/Glu-141His, Mk/Ile-133Lys/Glu-141His and Mk/His-135Leu/Glu-141His resulted in complete **toxin resistance** (>100,000-fold). The second approach, both novel and complementary, was to gain DT **binding** and sensitivity of Ms proHB-EGF by substitution(s) with the corresponding Mk residue(s). Surprisingly, the single **mutation** Ms/His-141Glu resulted in the gain of moderate DT sensitivity (> 260-fold). The double **mutation** Ms/Lys-133Ile/His-141Glu and the triple **mutation** Ms/Lys-133Ile/Leu-135His/His-141Glu resulted in a progressive gain in **toxin** sensitivity (> 4700-fold and > 16,000-fold respectively) and affinity. This triple mutant cell line is essentially as sensitive (IC50 = 3.1 ng ml-1) as the highly **toxin**-sensitive monkey Vero cell line (IC50 = 4 ng ml-1), indicating that these three Mk residues enable the Ms proHB-EGF to act as a fully functional DT receptor. Taken together, these results indicate that Glu-141 plays the most critical role in DT **binding** and sensitivity and that two additional amino acid residues, Ile-133 and His-135, also play significant roles.

L11 ANSWER 145 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:369335 CAPLUS
DOCUMENT NUMBER: 131:126599
TITLE: Aggregation of Bacillus thuringiensis Cry1A toxins upon **binding** to target insect larval midgut vesicles
AUTHOR(S): Aronson, Arthur I.; Geng, Chaoxian; Wu, Lan
CORPORATE SOURCE: Department of Biological Sciences, Purdue University, West Lafayette, IN, 47907, USA
SOURCE: Applied and Environmental Microbiology (1999), 65(6), 2503-2507
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB During sporulation, Bacillus thuringiensis produces crystalline inclusions comprised of a mixture of δ-endotoxins. Following ingestion by insect larvae, these inclusion proteins are solubilized, and the protoxins are converted to toxins. These **bind** specifically to receptors on the surfaces of midgut apical cells and are then incorporated into the membrane to form ion channels. The steps required for **toxin** insertion into the membrane and possible oligomerization to form a channel have been examined When bound to vesicles from the midguts of Manduca sexta larvae, the Cry1Ac **toxin** was largely **resistant** to digestion with protease K. Only about 60 amino acids were removed from the Cry1Ac amino terminus, which included primarily helix α1. Following incubation of the Cry1Ab or Cry1Ac toxins with vesicles, the prepns. were solubilized by relatively mild conditions, and the **toxin** antigens were analyzed by immunoblotting. In both cases, most of the **toxin** formed a large, antigenic aggregate of ca. 200 kDa. These **toxin** aggregates did not include the **toxin** receptor aminopeptidase N, but interactions with other vesicle components

were not excluded. No oligomerization occurred when inactive toxins with **mutations** in amphipathic helixes (α5) and known to insert into the membrane were tested. Active toxins with other **mutations** in this helix did form oligomers. There was one exception; a very active helix α5 mutant **toxin** bound very well to membranes, but no oligomers were detected. Toxins with **mutations** in the loop connecting helixes α2 and α3, which affected the irreversible **binding** to vesicles, also did not oligomerize. There was a greater extent of oligomerization of the Cry1Ac **toxin** with vesicles from the Heliothis virescens midgut than with those from the M. sexta midgut, which correlated with observed differences in toxicity. Tight **binding** of virtually the entire **toxin** mol. to the membrane and the subsequent oligomerization are both important steps in toxicity.

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 160 OF 180 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1995:930102 CAPLUS

DOCUMENT NUMBER: 123:330219

TITLE: Two subsites in the **binding** domain of the acetylcholine receptor: An aromatic subsite and a proline subsite

AUTHOR(S): Kachalsky, Sylvia G.; Jensen, Bo S.; Barchan, Dora; Fuchs, Sara

CORPORATE SOURCE: Dep. Chemical Immunology, Weizmann Inst. Science, Rehovot, 76100, Israel

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1995), 92(23), 10801-5
CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The ligand **binding** site of the nicotinic acetylcholine receptor (AcChoR) is localized in the α-subunit within a domain containing the tandem Cys-192 and -193. By analyzing the **binding**-site region of AcChoR from animal species that are **resistant** to α-neurotoxins, the authors have previously shown that for residues in this region, at positions 187, 189, 194, and 197, differ between animals sensitive (e.g., mouse) and **resistant** (e.g., mongoose and snake) to α- **bungarotoxin** (α-BTX). In the present study, the authors performed site-directed mutagenesis on a fragment of the mongoose AcChoR α-subunit (residues 122-205) and exchanged residues 187, 189, 194, and 197, either alone or in combination, with those present in the mouse α-subunit sequence. Only the mongoose fragment in which all four residues were **mutated** to the mouse ones exhibited α-BTX **binding** similar to that of the mouse fragment. The mongoose double **mutation** in which Leu-194 and His-197 were replaced with proline residues, which are present at these positions in the mouse AcChoR and in all other **toxin binders**, bound α-BTX to ≈60% of the level of **binding** exhibited by the mouse fragment. In addition, replacement of either Pro-194 or -197 in the mouse fragment with serine and histidine, resp., markedly decreased α-BTX **binding**. All other **mutations** resulted in no or just a small increase in α-BTX **binding**. These results have led the authors' to propose two subsites in the **binding** domain for α-BTX: the proline subsite, which includes Pro-194 and -197 and is critical for α-BTX **binding**, and the aromatic subsite, which includes amino acid residues 187 and 189 and dets. the extent of α-BTX **binding**.

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